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Research report

Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism

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Abstract

Idiopathic Parkinson's disease (PD) affects 2% of adults over 50 years of age. PD patients demonstrate a progressive loss of dopamine neurons in the substantia nigra pars compacta (SNpc). One model that recapitulates the pathology of PD is the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Here we show that exposure to an enriched environment (EE) (a combination of exercise, social interactions and learning) or exercise alone during adulthood, totally protects against MPTP-induced Parkinsonism. Furthermore, changes in mRNA expression would suggest that increases in glia-derived neurotrophic factors, coupled with a decrease of dopamine-related transporters (e.g. dopamine transporter, DAT; vesicular monoamine transporter, VMAT2), contribute to the observed neuroprotection of dopamine neurons in the nigrostriatal system following MPTP exposure. This non-pharmacological approach presents significant implications for the prevention and/or treatment of PD.
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Theme: Disorders of the nervous system
Topic: Degenerative disease, Parkinson's disease

Keywords: MPTP; Exercise; GDNF; Neurotrophic factors; Mouse

1. Introduction

Epidemiological studies have suggested that exposure to environmental toxins provides an increased risk to a number of neurological disorders [62,72]. Perhaps the most studied of these is PD, which affects 2% of adults over 50 years of age and is characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). Current treatments for PD are numerous, the most widely used being DA-replacement therapy. Amelioration of PD symptoms through this method is transient and for this reason, several non-pharmacological methods have been developed in an attempt to permanently reverse the symptoms of SNpc cell loss including transplantation of DA cells [25] and destruction of cerebral motor pathway nuclei [58]. None of the current therapies are aimed at preventing the disorder and some may exacerbate the condition [25]. Another problem in treating PD is that a majority of the SNpc neurons are lost at the onset of visible symptoms [6]. However, since PD generally presents in the 6th decade of life, identification of a mechanism or therapy that slows or ameliorates the cell loss for several years could, due to actuarial realities, eliminate symptomatic PD in many individuals.

One of the best models for generating experimental PD is the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes a specific loss of SNpc neurons identical to that seen in PD [42]. Exposure to MPTP has similar effects in many vertebrate species, including man and mouse. MPTP is metabolized by glial MAO-B to MPP^+. MPP^+ is transported into neurons through the dopamine transporter (DAT) where it interferes with Complex I respiration [44,55]. Once in cells, MPP^+ is sequestered into vesicles by the vesicular monoamine transporter (VMAT2), which may provide some cellular protection against this

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toxin [28,73]. Thus, the relative expression of DAT and VMAT2 within a neuron may determine its potential for survival from an exogenous toxin [50].

In addition to the genetic control of MPTP-induced SNpc neuron loss [38], several studies have shown that environmental factors can alter the prevalence and outcome of neurodegenerative disease [75]. Some of these can be experimentally recapitulated through exposure to a modified or "enriched" environment (EE) that incorporates social interactions, learning and exercise [43,45].

The complexity of an animal's environment has been shown to affect brain structure and function. EE exposure leads to increases in neuron size, dendrite length, synaptic density and spine number [3,11,14,17,21,22,35,36,80]. It has also been demonstrated that animals raised in an EE have increased neurotrophin levels [40,53,59,66].

In this study, we examine the role of environment in modulating SNpc cell loss following administration of MPTP. We show that introducing animals to an enriched environment as adults totally protects against MPTP-induced Parkinsonism. Within the EE, exercise appears to be the critical component. The likely mechanism for this neuroprotection is a significant increase in specific growth factors coupled with a down-regulation of dopamine-related transporters in the nigrostriatal system.

2. Materials and methods

2.1. Animal procedures

All mice used in this study were C57Bl/6J (Jackson Laboratories, Bar Harbor, ME). Animals were maintained in a temperature-controlled environment with free access to food and water and kept on a 12-h light/dark cycle; lights on at 7.00 am. All animal procedures were in compliance with St. Jude Children's Hospital Institutional guidelines and were approved by the SJCRH Institutional Animal Care and Use Committee.

2.2. Environment preconditioning

The animals were preconditioned in environments termed either (1) "Enriched" (14 mice/cage) (2) "Exercise" (4 mice/cage) (3) and standard (4–6 mice/cage). The "Enriched Environment" cages (1×1 m) consisted of two running wheels, nesting material and a system of interchangeable tunnels re-arranged on a weekly basis [43]. The "Exercise cages" consisted of a standard mouse colony cage containing a running wheel. Running wheels in the "enriched" and "exercise" cages" were wire meshed with a diameter of 12.7 cm. These wheels were freely available, but the individual animals activity levels were not monitored. In a separate experiment, the daily activity of individual female C57Bl/6J mice was measured using exercise wheels from Lafayette Instruments (Lafayette, IN, Model 80820).

For the experimental animals, female mice were born and raised in "standard" cages. At 2–3 months, these mice were removed from their home cage and placed into either an EE or exercise cage. The reason we used only female mice is that we wanted to eliminate any bias arising from the use of a single litter. Thus, the production of mixed litter cages was only possible by using female mice since it is well known that they exhibit decreased stress and aggressive behavior in a mixed population [60]. In a previous study, we showed that there was no difference in MPTP-sensitivity in male and female C57Bl/6J mice [38].

2.3. MPTP treatment

All animals injected with MPTP were between 5 and 7 months. Animals received MPTP (4×20 mg/kg/2-h intervals) (Research Biochemical International, Natick, MA) in a 5-ml/kg volume or vehicle (sterile saline) alone. For the time course studies, animals were injected s.c., while animals in the EE experiments were injected i.p. Seven days (a time corresponding to maximal SNpc cell loss [41]) and 14 days after MPTP administration animals were anesthetized with tribromoethanol (250 mg/kg (i.p.)) and transcardially perfused with 0.1 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Brains were removed, post-fixed overnight, processed and sectioned at 5 μm and cut throughout the SNpc and mounted onto polyionic microscope slides (Superfrost-plus, Fisher). In addition, a number of animals were anesthetized, decapitated and the SN and striatum (STR) dissected out and rapidly froze on dry ice and stored at −80 °C until RNA processing.

2.4. Immunohistochemistry

Standard immunohistochemical techniques were used to identify cells positive for tyrosine hydroxylase (TH) in the SNpc. Paraffin sections were stained with a polyclonal antibody specific for TH (1:250; Pel Freez, Rogers, AR). Immunopositive cells were subsequently visualized using a peroxidase-anti-peroxidase system (Vector Laboratories, CA) and DAB (KPL, Gaithersburg, MD). Slides were then counterstained with the Nissl stain Neutral Red, dehydrated through a graded series of alcohol, mounted in Permount and coverslipped.

2.5. Cell quantitation

The major neuron-type in the SNpc is dopaminergic and can be visualized by immunolabeling with tyrosine hydroxylase (Fig. 2A). In order to make sure that we counted all neurons in the SNpc and not just those exhibiting the phenotypic marker (TH), we counterstained every immunostained section with the Nissl marker Neutral Red. All cells in the SNpc having a the appearance of dopaminergic neurons (TH+Nissl) were counted as previously described using both stereological and 2D-measurement techniques [12,38]. As

previously reported, we found no significant difference in the final counts of SNpc that was technique-dependent.

2.6. Real-time PCR analysis

SN was dissected as previously described [70]. Briefly, animals are placed into CO_2 until unconscious and then their brains are rapidly dissected from the calvaria. Brains are placed into a rodent brain matrix (EM Sciences Model 69022) with marked cuts at 1-mm intervals. To dissect the SN, a 1-mm section corresponding to Bregma −2.70 to −3.70 [61] is removed and placed on ice. The cortical tissue and hippocampus are removed and then a ventrolateral piece of tissue is dissected using the medial lemniscus as the medial limit and the medial geniculate as the rostral limit. SN from both sides are dissected and placed into a 1.5-ml microfuge tube and frozen on dry ice. For the striatal sample, a 2-mm tissue section is dissected corresponding to Bregma +1.18 to −0.18 [61]. The striatal tissue is visualized by the presence of internal capsule fibers, dissected from the overlying cortical tissue and rapidly frozen in 1.5-ml microfuge tubes.

The RNA from SN and STR was isolated using a RNeasy mini kit (Qiagen, Valencia, CA) and stored at −80 °C for further processing. For the real-time PCR reaction, samples were run in 96-well optical reaction plates with optical adhesive covers using the Taqman® One-Step RT-PCR reaction on an ABI Prism 7900 Sequence Detection System using VIC™-labeled 18S ribosomal RNA probe as an internal control (Applied Biosystems, Forest City, CA). Primers and probes for brain-derived neurotrophic factor (accession no. X55573), glia-derived neurotrophic factor (accession no. NM010275), dopamine transporter (accession no. AH003328), vesicular monoamine transporter2 (accession no. AJ555564), insulin-like growth factor I (accession no. AF440694) and IGFI receptor (accession no. AF056187) were designed using Primer Express software (version 1.5, Applied Biosystems) and were labeled with the reporter dye FAM and the quencher TAMRA. Primer sequences used to amplify each gene were:

BDNF	CCATAAGGACGCGGACTTGT (F-primer)
	GAGGCTCCAAAGGCACTTGA (R-primer)
	CACTTCCCGGGTGATGCTCAGCA (FAM-labeled probe)
GDNF	CCGTGGCCCTAAAGACCC (F-primer)
	TCCACTTCCCAGTCCTGCA (R-primer)
	CGTTTCGCATGGTTCCACACCCC (FAM-labeled probe)
IGF1	CTACAAAAGCAGCCCGCTCT (F-primer)
	CTTCTGAGTCTTGGGCATGTCA (R-primer)
	TCCGTGCCCAGCGCCACA (FAM-labeled probe)
IGF1R	AGAGCAGTGACTCGGATGGC (F-primer)
	TGAAGCCTGAGGGACACTCC (R-primer)
	CGTTATCCACGACGATGAGTGCATGC (FAM-labeled)
DAT	TCGATGCCGCCACCC (F-primer)
	AGGCAATCAGCACCCCAA (R-primer)
	TGTGCTTCTCCCTTGGCGTTGGG (FAM-labeled probe)
VMAT2	TGGATGATGGAGACCATGTGTT (F-primer)
	TCCTGCCAGCGAGCATC (R-primer)
	CCGAAAGTGGCAGCTGGGCG (FAM-labeled probe)

Relative levels of mRNA were determined by comparing growth factor and monoamine transporter expression with control 18S mRNA levels in each sample. Each data point was performed in triplicate and replicated at least three times.

2.7. Measurement of striatal MPP^+ levels

Two groups of adult C57BL/6 mice (n=4 per group), one group raised from 3 to 6 months in an enriched environment (EE), and a second group raised in a standard environment, received a single i.p. injection of 20 mg/kg MPTP hydrochloride (Sigma). Two hours after the injection, mice were sacrificed the striatum of each mouse was dissected-out on ice and homogenized in 10 volumes of ice-cold 0.1 M perchloric acid (Fisher). The homogenate was centrifuged at 12,000×g for 15 min at 4°C. The supernatants were filtered in 0.2-μm filters for 2 min at 8000×g, and 100 μl of the supernatant was injected into a HPLC system equipped with an autosampler (Shimadzu), a programmable solvent module (Shimadzu), a Nucleosil 100-5 C18 (4×125 mm, 5 μm) column (Agilent Technologies), and a UV detector. The mobile phase consisted of 50 μM sulfuric acid (Sigma), 10% acetonitrile, 90% water, and 75 mM triethylamine (Sigma) adjusted to final pH 2.3 (run under isocratic conditions, at 1.0 ml/min). The UV detector was set to 295 nm for MPP^+ detection, as previously described [24]. The concentrations of MPP^+ were determined by running known amounts of MPP^+ iodide (Sigma) dissolved in 0.1 M perchloric acid and extrapolating from a standard curve.

2.8. Statistical analyses

Statistical comparisons were made using ANOVA followed by Bonferroni post-hoc comparisons when applicable (Statview, SAS software).

3. Results

3.1. The effects of placing adult animals into an enriched environment on the MPTP-induced SNpc cell death

To determine whether exposure to an EE had any effect on the baseline number of neurons in the SNpc, we compared adult C57Bl/6J animals from standard housing (control) with those placed into an EE during adulthood. We found that the different environments had no effect on SNpc neuron number (Fig. 1A). To determine if adult animals preconditioned for 3 months in an EE had an altered cellular response to MPTP, we examined the number of SNpc dopamine neurons 7 days following administration of this toxin. The number of SNpc cells in these conditions is also shown in Fig. 1A. To determine if the lack of cell death in the EE condition was due to protection or just a delay in cell death, we examined SNpc cell number 14 days following

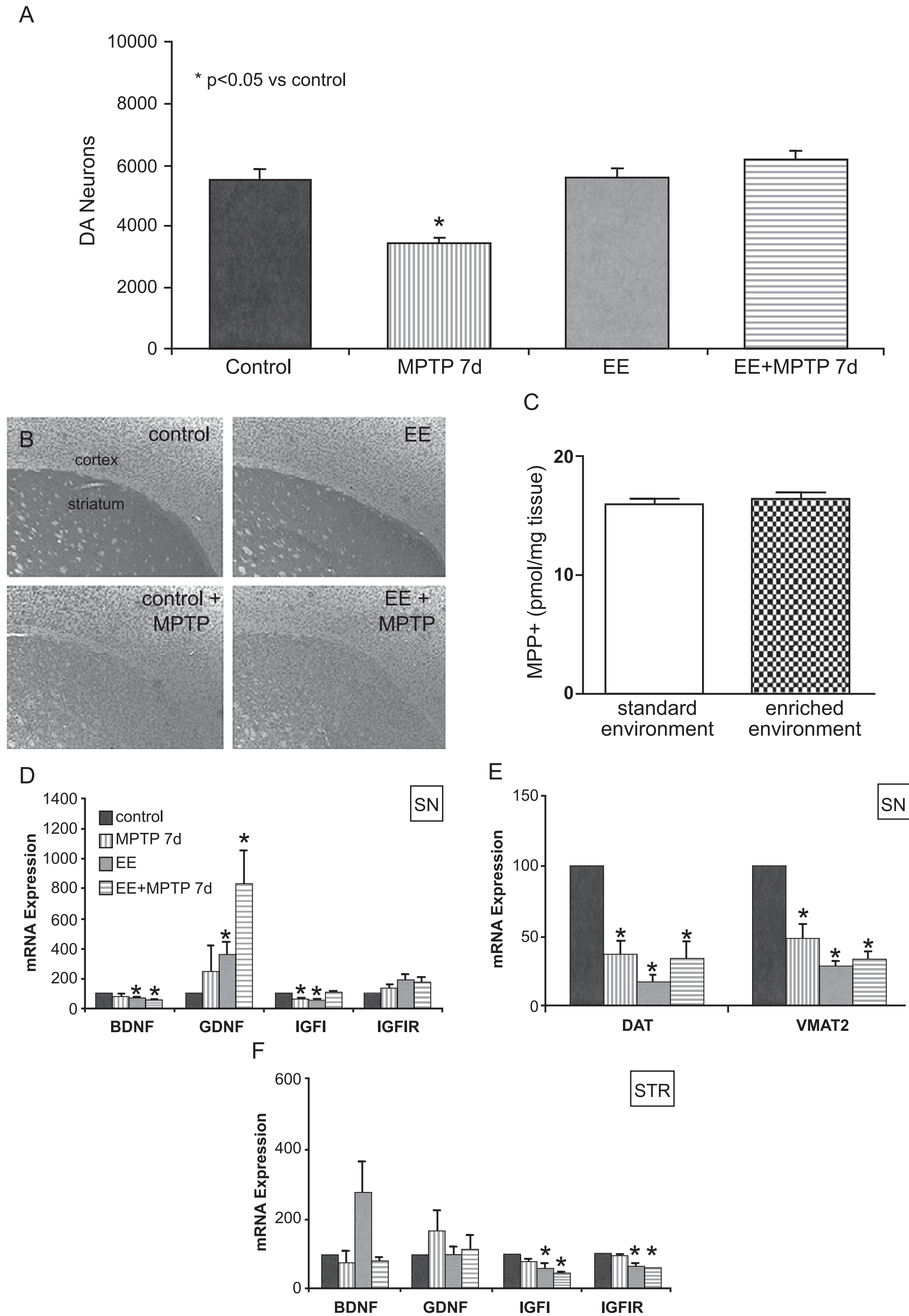

Fig. 1. Analysis of tyrosine hydroxylase (TH)-positive neurons and mRNA expression in animals placed into an enriched environment (EE) during adulthood. (A) Graph showing the number of dopamine neurons in the substantia nigra pars compacta (SNpc). (B) TH-staining in the STR illustrating equipotent effects of MPTP on control and EE animals. (C) Levels of striatal MPP^+ determined by HPLC analysis 2 h following i.p. injection of MPTP in C57Bl/6 mice from standard and EE environments. (D,E) Relative expression of growth factors and monoamine transporters in the SN. (F) Relative expression of growth factors in the striatum (STR). For each group, n=3–6.

MPTP. There was no difference in cell number at 14 days post-MPTP compared to that seen 7 days-post MPTP (data not shown). This suggests that the lack of cell death in mice placed in an EE is protected from the toxin rather than having a delay in the process of cell death.

Since the administration of MPTP in our studies was given systemically, it was possible that the lack of an effect seen following preconditioning in the EE was due to a change in MPTP metabolism. HPLC analysis showed that conversion of MPTP to MPP^+ in the striatum was no different in animals raised in standard and EE conditions (Fig. 1C), eliminating peripheral detoxification as a potential source of the protection. In addition, we show equal loss of the dopaminergic nigrostriatal terminals in both conditions (Fig. 1B). These two experiments suggest that any neuronal protection occurs via a central protection mechanism rather than by differences in peripheral detoxification or central MPTP metabolism.

3.2. mRNA expression of growth factors and monoamine transporters following placement into an EE

Animals placed into an EE during adulthood showed a 350% increase in GDNF expression in the SN compared to animals raised in standard housing. When animals preconditioned to an EE received MPTP, there was an additional 180% increase from EE animals alone in GDNF expression (total increase of 750% compared to control) compared to

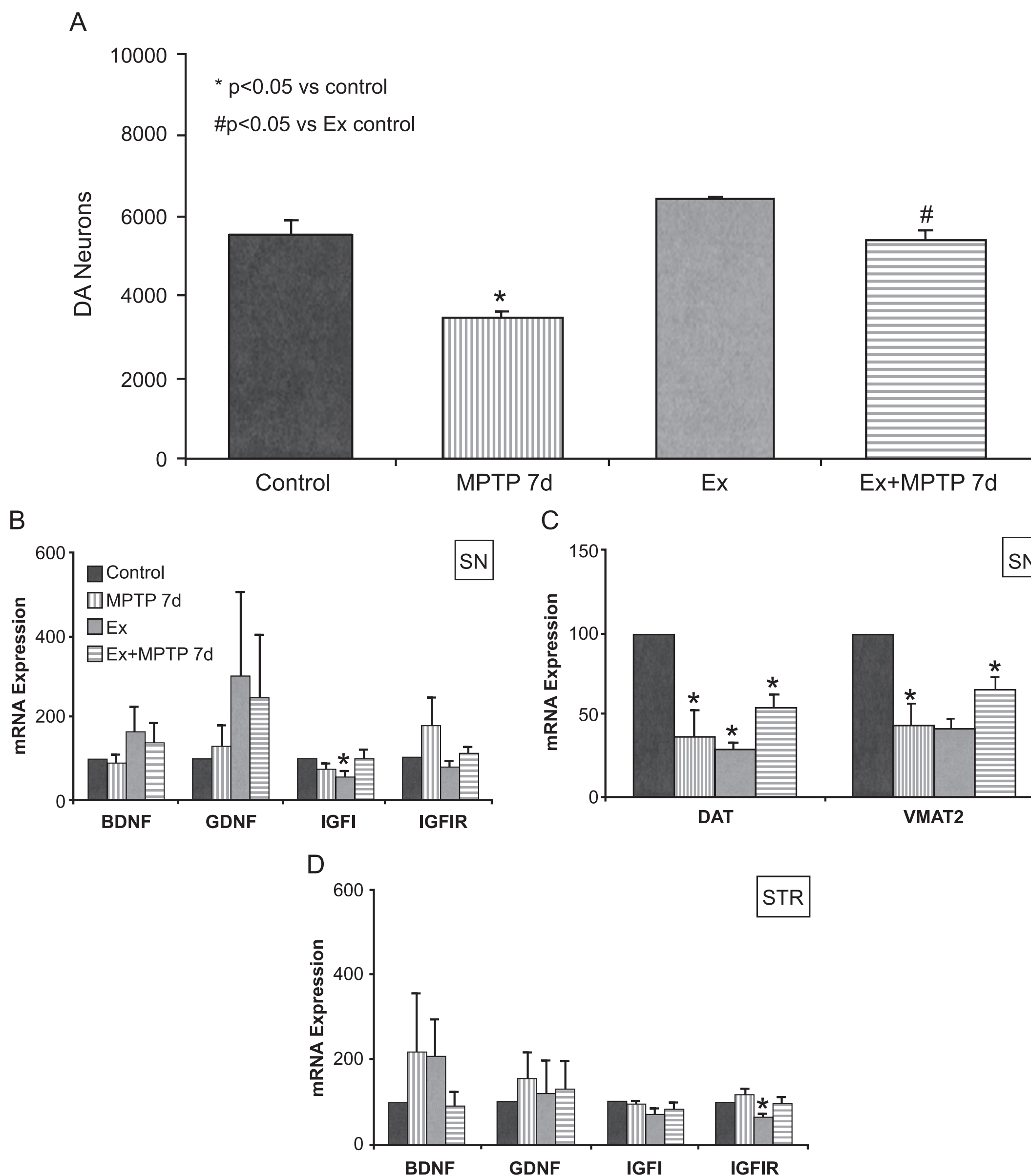

Fig. 2. Analysis of tyrosine hydroxylase (TH)-positive neurons and mRNA expression in animals placed into an "exercise" cage during adulthood. (A) Graph showing the number of dopamine neurons in the substantia nigra pars compacta (SNpc). (B,C) Relative expression of growth factors and monoamine transporters in the SN and (D) striatum (STR). For each group, n=3–6.

animals raised in standard housing. In addition, animals raised in an EE had a ~50% reduction in the expression of IGFI compared to control animals. Unexpectedly, we also found a significant decrease (32%) in BDNF expression in SN of animals exposed to an EE compared to control animals (Fig. 1D).

We also examined the effect of an EE on the expression of the monoamine transporters, DAT and VMAT2. Compared to mice raised in standard housing, the administration of MPTP resulted in a 63% reduction in the DAT expression in the SN. In a similar vein, the expression of DAT was decreased by 83% in mice raised in an EE compared to animals raised in standard housing. We also found a 53% decrease in nigral VMAT2 mRNA expression in the control+MPTP mice and a significant 72% decrease in the EE group compared to animals raised in standard housing (Fig. 1E).

Unlike the SN, only a few changes were found in mRNA expression within the STR. Here, we found a significant 41% reduction in IGF-1 and 35% in IGFI receptor expression as a result of exposure to an EE. A second neurotrophin, BDNF, also showed large changes in expression (175% increase) in mice exposed to an EE compared to control mice, although the variability in expression of this neurotrophin (±87%) precluded statistical significance (Fig. 1F).

3.3. The effects of exercise on the MPTP-induced SNpc cell death

To determine if exercise alone was sufficient to convey neuroprotection from MPTP, we placed 3-month-old adult animals raised in standard cages into mouse colony cages containing a running wheel. Although animals in the EE cages were not monitored, a separate experiment showed that female C57Bl/6J mice ran an average distance/day of 12.54±0.65 km. This is similar to the running distance previously reported for C57Bl/6 mice [47]. We found that exercise-alone protected against MPTP-induced toxicity, although the amount of protection was slightly reduced compared to the EE mice (16% loss versus 0% loss) (compare Figs. 1A to 2A). The difference in percentage of cell loss between the control+MPTP and exercise only+MPTP was significantly different ($p \leq 0.001$), demonstrating that exercise did have neuroprotective effects (Fig. 2A).

3.4. mRNA expression of growth factors and monoamine transporters following adult exposure to exercise

In animals given free access to exercise, there was a 35% reduction of IGF-1 in SN (Fig. 2B). In addition, there was an ~250% increase in the mean mRNA expression for GDNF in the SN as a result of exercise training, although no statistical significant was observed (Fig. 2B–C). In the STR, there was a 37% reduction of mRNA encoding for the IGF-1 receptor as a result of preconditioning with exercise (Fig. 2D). In addition, similar to GDNF expression in the SN, there was a trend showing a ~50% increase in expression of BDNF mRNA in the STR as a result of MPTP and exercise training (Fig. 2D).

In addition to the neurotrophins, we examined the effect of exercise-alone on the expression of the monoamine transporters, DAT and VMAT2. We found a 64% reduction in the mRNA expression for DAT in the SN in control+MPTP mice and a 70% reduction in the exercise-only control group compared to animals raised in a standard environment (Fig. 2C). We also observed a 58% decrease in nigral VMAT2 mRNA expression in the control+MPTP mice and a 59% decrease in the exercise-only group compared to animals raised in a standard caging (Fig. 2C).

4. Discussion

In this paper, we demonstrate that alteration of an animal's environment as an adult, absent of any exogenous pharmacological manipulations, can change its sensitivity to a neurotoxic agent that has been shown, in humans, to recapitulate the pathology of idiopathic Parkinson's disease (PD). This protection is not due to peripheral detoxification as it has been shown by HPLC that equal levels of the toxin (MPP^+) are present in the brain of both control and animals raised in an EE. The second measure of cell protection was obtained by directly examining cell number in the SNpc. In performing cell counts, we count both TH+ plus Nissl neurons in the SNpc. By counting both populations, we insure that the observed changes caused by MPTP are from actual cell loss and not just the reduction of the phenotypic marker such as striatal dopamine levels [2,78]. Another reason we did not measure dopamine levels directly is that i.p. injection of MPTP results a more mild anatomical lesion than is typically seen in human PD (39% cell loss versus upwards of 60% cell loss, respectively). In the acute injection paradigm of MPTP, the neuronal loss is maximal at 7 days and does not appear to significantly progress at 2 weeks. Furthermore, we have evidence (unpublished research) that the lesion is stable at 3 months following administration of MPTP lesion. This pattern of loss is quite different than has been reported for striatal dopamine loss following MPTP, where administration to young mice (2–3 months) causes is a rapid loss of DA followed by a significant recovery [23,67].

In this study, we examine changes in cell number and gene expression after administration of MPTP in mice housed in different environments. When analyzing this type of data, one must be careful to consider that the observed changes may reflect either physiological alterations in cell function, such as are seen following recovery from an injury [20], or might simply be due to a lower cell number following a period of cell death. We examined mRNA levels by real-time PCR since we were using very small samples

(individual SN) that do not contain enough cells for Northern or Western blot analysis. However, previous studies of mRNA and protein expression of neurotrophins have shown a concurrence between these measurements [31,32].

In this study, we find a 39% neuron loss in the SNpc following MPTP treatment. If gene expression changes were simply due to cell loss, we would expect to see a 40% loss of mRNA expression, or perhaps slightly lower due to compensatory mechanisms. The only instance that we observe this type of relationship is with IGFI in the striatum (Fig. 1F) and for this reason, we do not believe that the changes seen in this factor are responsible for the observed neuroprotection. All of the other changes seen in gene expression are far greater than the observed cell loss (i.e. DAT and VMAT in EE condition) or are in the opposite direction, i.e. they are higher in the MPTP conditions (i.e. GDNF in EE condition).

Once in the CNS, the protection of dopaminergic neurons following exposure to MPTP can occur at various points in the toxicity cascade. The protection of neurons can be due to alterations in toxin availability, changes in the expression of neuroprotective factors such as the neurotrophins, or interference with activation of the cell death pathways. Other labs have reported that exposure to an EE can confer protection in a number of other experimental paradigms that utilize different mechanisms of cell death [18,33,76]. For this reason, we did not think that the protection conferred by the EE was due to interference with the process of cell death. Instead, we focused on examination of genes that either confer toxin availability or neurotrophins that have been shown to be protective to SN neurons or are responsive to alterations of environment [34,49,54,79,84].

One of the initial events in MPTP toxicity is the transport of MPP^+ into the cell through the DAT. DAT plays a significant role in MPTP toxicity since DAT-null animals are completely protected from MPP^+-induced neuronal damage [5,27], while mice over-expressing the transporter show increased sensitivity to the toxin [19]. In this study, we find that the reduction of mRNA expression of SN DAT following exposure to an EE or exercise alone compared to mice raised in a standard environment exceeds the amount of cell loss. In addition, the reduction in SN DAT mRNA levels that we observed was similar to that seen in the striatum of EE mice using both radioligand binding to DAT or in situ grain counts [4]. A recent paper demonstrated that mice that run for 1 month on a treadmill, even with a much lower dosage of MPTP (30 mg/kg total versus 80 mg/kg total in our study) have a significant loss of striatal DAT protein [23]. Taken together, this suggests that one component of the neuroprotection conferred by environmental enrichment is the exclusion of toxins by down-regulation of the DAT. On the other hand, since MPTP is converted to MPP^+ within 2 h and enters into the striatum equally in animals raised in an EE, it is likely that the reduction in DAT we observe is not sufficient to prevent entry of a toxic level of MPP^+.

Once in the cell, MPP^+ can either enter the mitochondria where it is known to interfere with the function of complex I of the electron transport chain [51] or it can be actively sequestered into protective cytoplasmic vesicles by the vesicular monoamine transporter [50]. Previous studies have shown that reduction in VMAT2 makes mice more sensitive to the effects of MPTP [73]. In this study, however, we find a loss of VMAT mRNA expression; yet still observe a neuroprotection. Based on our results, it might be hypothesized that the sequestration of MPP^+ into cytoplasmic vesicles is not a critical point of neuroprotection in our model. However, this is unlikely and a more probable alternative is that the levels of VMAT2 expression need to be examined in the context of other changes in the cell. Miller et al. [50] have suggested that the ratio of DAT/VMAT2 can be used as a predictor of neuron susceptibility to MPTP. In this study, we find that both environmental enrichment and exercise increase the ratio of VMAT/DAT mRNA levels based on real time PCR quantitation. Thus, even though mice exposed to an EE or exercise alone have reduced levels of both DAT and VMAT2, the ratio following EE or exercise shifts in favor of VMAT2, which has been shown to provide an increased level of protection [27,28]. More simply stated, the decrease in VMAT2 mRNA, which would predict less sequestration of MPP^+ and hence a greater susceptibility to MPP^+, appears to be being compensated by the decreased entry of the toxin into the cell secondary to decreased DAT mRNA expression.

Once in the cell, MPP^+ is transported into the mitochondria where it interferes with Complex I. The energy depletion that follows from this blockade results in numerous effects including the release of dopamine from intracellular stores [64]. Once into the extracellular space, dopamine becomes highly adducted [57], induces genes known to be responsive to free radicals [68], and causes cellular damage [74]. Many studies have shown that neuronal growth factors are capable of protecting cells against oxidative stress [83]. In our study, we found significant changes in two neurotrophins: a down-regulation of IGF-1 (and IGF1R) and an up-regulation of GDNF.

Previous in vitro studies have shown that IGF-1 can protect dopaminergic neurons from oxidative stress [39]. This protection has also been noted in vivo, where it was shown that exercise can up-regulate the mount of circulating IGF-1 that is subsequently taken up into neurons [7]. Our studies do not dispute the effects of IGF-1, but do suggest that any alterations in expression may be peripherally derived, rather than result from any intrinsic CNS effect [82].

There is ample evidence demonstrating that exogenous GDNF protects against MPTP-induced neurotoxicity in C57Bl/6 mice, rhesus monkeys, and marmosets [9,13,15,29] and against 6-hydroxydopamine-induced cell death in rats [65]. Since GDNF has been shown to have

protective effects in animal models of PD, clinical trials using this trophic factor have been performed in PD patients [30,56]. The use of GDNF for clinical treatment is hampered by its poor bioavailability, although a number of delivery systems including implantable pumps [37] and viral vectors [49] are being developed to alleviate this problem. In this paper, we describe a non-invasive mechanism that increases expression of GDNF in the anatomic regions important for PD.

Interestingly, our study did not find a significant increase in BDNF gene expression as has been reported in several studies following an environmental enrichment or exercise [4,66]. One significant difference in this study is that in previous studies, animals were introduced into the EE or exercise regimens at early ages. In this study, mice were introduced into the EE or exercise cages as adults. It is possible that the dependence of the SNpc neurons on GDNF rather than BDNF reflects a developmental switch in neurotrophin dependence [8]; and also highlights the potential of this neurotrophic factor in adult therapeutic procedures.

Our use of real-time PCR allows for an accurate assessment of mRNA levels from very small pieces of tissue. In this study, we were able to examine the effects of environmental enrichment in individual substantia nigra and striatum, without contamination from other parts of the midbrain or forebrain. While the CNS regions examined were circumspect, we do not know if the observed mRNA changes originate from the neuronal or glial compartments (or a combination of the two). Previously we have shown that the strain-dependent differences in MPTP and MPP^+ toxicity in mice are modulated by glial cells [69]. In addition, it has been shown that glial cells are capable of producing the growth factors that we have implicated in neuronal protection [26,52]. Thus, it is possible that environmental enrichment alters not only the neuronal but also the glial microenvironment.

Another component of our study examined the relative contributions of exercise alone versus exercise in combination with increased “learning” and “social” interactions (the total EE). Previous studies using a variety of lesions have suggested that exercise is the causal factor that leads to neuroprotection [48,54,59,71,77,81]. We found that C57Bl/6J mice given free access to running wheels were protected against MPTP-induced toxicity, although the amount of protection was slightly reduced compared to the EE mice (16% loss versus 0% loss). Thus, in this study, we find that exercise-alone can provide significant, but not complete neuroprotection from exposure to MPTP. One possible explanation for this discrepancy is that our studies were done in mice placed into an EE or exercise-alone condition as adults, while the other studies examining the role of exercise were done with animals exposed to these conditions at earlier ages.

The pathologies seen following administration of MPTP, which were first described in humans [46], dramatically mirror that seen in idiopathic PD. Although the lesion in mice following MPTP is smaller than seen in humans, their similarities suggests that the mechanism of neuronal loss in MPTP-induced and idiopathic PD may utilize similar biochemical processes [16]. In humans, it is well known that exercise can slow the progression of PD symptomatology [1,10,63]. However, it has been thought that this benefit resulted from an increase in muscle mass and strength. Although our final lesion is less than that seen at end-stage human PD, we do find complete protection of the SNpc neurons. Since symptoms of human PD do not occur until there is a loss of approximately 60% of the neuron and a 70% loss of striatal dopamine, any significant protection (even of 40% cell loss, as we find) would have the potential to provide a significant clinical effect. For this reason, it can be suggested that exercise, in concert with continued mental activity and social interactions, may provide a non-invasive, non-pharmaceutical mechanism to protect against the onset of Parkinsonian symptoms.

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References

[1] J. Baatile, W.E. Langbein, F. Weaver, C. Maloney, M.B. Jost, Effect of exercise on perceived quality of life of individuals with Parkinson’s disease, J. Rehabil. Res. Dev. 37 (2000) 529–534.

[2] S. Barc, G. Page, L. Barrier, L. Garreau, D. Guilloteau, B. Fauconneau, S. Chalon, Relevance of different striatal markers in assessment of the MPP^+-induced dopaminergic nigrostriatal injury in rat, J. Neurochem. 80 (2002) 365–374.

[3] C. Beaulieu, M. Colonnier, Effect of the richness of the environment on the cat visual cortex, J. Comp. Neurol. 266 (1987) 478–494.

[4] E. Bezard, S. Dovero, D. Belin, S. Duconger, V. Jackson-Lewis, S. Przedborski, P.V. Piazza, C.E. Gross, M. Jaber, Enriched environment confers resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and cocaine: involvement of dopamine transporter and trophic factors, J. Neurosci. 23 (2003) 10999–11007.

[5] E. Bezard, C.E. Gross, M.C. Fournier, S. Dovero, B. Bloch, M. Jaber, Absence of MPTP-induced neuronal death in mice lacking the dopamine transporter, Exp. Neurol. 155 (1999) 268–273.

[6] T. Brucke, S. Asenbaum, W. Pirker, S. Djamshidian, S. Wenger, C. Wober, C. Muller, I. Podreka, Measurement of the dopaminergic degeneration in Parkinson’s disease with [^{123}I] beta-CIT and SPECT. Correlation with clinical findings and comparison with multiple system atrophy and progressive supranuclear palsy, J. Neural Transm., Suppl. 50 (1997) 9–24.

[7] E. Carro, J.L. Trejo, S. Busiguina, I. Torres-Aleman, Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy, J. Neurosci. 21 (2001) 5678–5684.

[8] A. Chalazonitis, T.P. Rothman, J. Chen, M.D. Gershon, Age-dependent differences in the effects of GDNF and NT-3 on the development of neurons and glia from neural crest-derived precursors immunose-

lected from the fetal rat gut: expression of GFRalpha-1 in vitro and in vivo, Dev. Biol. 204 (1998) 385–406.

[9] F.C. Cheng, D.R. Ni, M.C. Wu, J.S. Kuo, L.G. Chia, Glial cell line-derived neurotrophic factor protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity in C57BL/6 mice, Neurosci. Lett. 252 (1998) 87–90.

[10] C.L. Comella, G.T. Stebbins, N. Brown-Toms, C.G. Goetz, Physical therapy and Parkinson's disease: a controlled clinical trial, Neurology 44 (1994) 376–378.

[11] T.A. Comery, C.X. Stamoudis, S.A. Irwin, W.T. Greenough, Increased density of multiple-head dendritic spines on medium-sized spiny neurons of the striatum in rats reared in a complex environment, Neurobiol. Learn Mem. 66 (1996) 93–96.

[12] R. Cook, L. Lu, J. Gu, R.W. Williams, R.J. Smeyne, Identification of a single QTL, Mptp1, for susceptibility to MPTP-induced substantia nigra pars compacta neuron loss in mice, Mol. Brain Res. 110 (2003) 279–288.

[13] S. Costa, M.M. Iravani, R.K. Pearce, P. Jenner, Glial cell line-derived neurotrophic factor concentration dependently improves disability and motor activity in MPTP-treated common marmosets, Eur. J. Pharmacol. 412 (2001) 45–50.

[14] R.A. Cummins, P.J. Livesey, J.A. Bell, Cortical depth changes in enriched and isolated mice, Dev. Psychobiol. 15 (1982) 187–195.

[15] I. Date, M. Aoi, S. Tomita, F. Collins, T. Ohmoto, GDNF administration induces recovery of the nigrostriatal dopaminergic system both in young and aged parkinsonian mice, NeuroReport 9 (1998) 2365–2369.

[16] T. Dawson, A. Mandir, M. Lee, Animal models of PD: pieces of the same puzzle? Neuron 35 (2002) 219–222.

[17] M.C. Diamond, M.R. Rosenzweig, E.L. Bennett, B. Lindner, L. Lyon, Effects of environmental enrichment and impoverishment on rat cerebral cortex, J. Neurobiol. 3 (1972) 47–64.

[18] R.C. Dodel, Y. Du, K.R. Bales, Z.D. Ling, P.M. Carvey, S.M. Paul, Peptide inhibitors of caspase-3-like proteases attenuate 1-methyl-4-phenylpyridinum-induced toxicity of cultured fetal rat mesencephalic dopamine neurons, Neuroscientist 86 (1998) 701–707.

[19] D.M. Donovan, L.L. Miner, M.P. Perry, R.S. Revay, L.G. Sharpe, S. Przedborski, V. Kostic, R.M. Philpot, C.L. Kirstein, R.B. Rothman, C.W. Schindler, G.R. Uhl, Cocaine reward and MPTP toxicity: alteration by regional variant dopamine transporter overexpression, Mol. Brain Res. 73 (1999) 37–49.

[20] R. Dubner, M.A. Ruda, Activity-dependent neuronal plasticity following tissue injury and inflammation, TINS 15 (1992) 96–103.

[21] C.J. Faherty, D. Kerley, R.J. Smeyne, A Golgi-Cox morphological analysis of neuronal changes induced by environmental enrichment, Dev. Brain Res. 141 (2003) 55–61.

[22] B.A. Fiala, J.N. Joyce, W.T. Greenough, Environmental complexity modulates growth of granule cell dendrites in developing but not adult hippocampus of rats, Exp. Neurol. 59 (1978) 372–383.

[23] B.E. Fisher, G.M. Petzinger, K. Nixon, E. Hogg, S. Bremmer, C.K. Meshul, M.W. Jakowec, Exercise-induced behavioral recovery and neuroplasticity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse basal ganglia, J. Neurosci. Res. 77 (2004) 378–390.

[24] F. Fornai, G. Battaglia, M. Gesi, F.S. Giorgi, F. Orzi, F. Nicoletti, S. Ruggieri, Time-course and dose–response study on the effects of chronic L-DOPA administration on striatal dopamine levels and dopamine transporter following MPTP toxicity, Brain Res. 887 (2000) 110–117.

[25] C.R. Freed, P.E. Greene, R.E. Breeze, W.Y. Tsai, W. DuMouchel, R. Kao, S. Dillon, H. Winfield, S. Culver, J.Q. Trojanowski, D. Eidelberg, S. Fahn, Transplantation of embryonic dopamine neurons for severe Parkinson's disease, N. Engl. J. Med. 344 (2001) 710–719.

[26] W.J. Friedman, I.B. Black, D.R. Kaplan, Distribution of the neurotrophins brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 in the postnatal rat brain: an immunocytochemical study, Neuroscience 84 (1998) 101–114.

[27] R.R. Gainetdinov, F. Fumagalli, S.R. Jones, M.G. Caron, Dopamine transporter is required for in vivo MPTP neurotoxicity: evidence from mice lacking the transporter., J. Neurochem. 69 (1997) 1322–1325.

[28] R.R. Gainetdinov, F. Fumagalli, Y.M. Wang, S.R. Jones, A.I. Levey, G.W. Miller, M.G. Caron, Increased MPTP neurotoxicity in vesicular monoamine transporter 2 heterozygote knockout mice, J. Neurochem. 70 (1998) 1973–1978.

[29] D.M. Gash, Z. Zhang, G. Gerhardt, Neuroprotective and neurorestorative properties of GDNF, Ann. Neurol. 44 (1998) S121–S125.

[30] S.S. Gill, N.K. Patel, G.R. Hotton, K. O'Sullivan, R. McCarter, M. Bunnage, D.J. Brooks, C.N. Svendsen, P. Heywood, Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson's disease, Nat. Med. 9 (2003) 589–595.

[31] F. Gomez-Pinilla, L. Dao, V. So, Physical exercise induces FGF-2 and its mRNA in the hippocampus, Brain Res. 764 (1997) 1–8.

[32] F. Gomez-Pinilla, Z. Ying, P. Opazo, R.R. Roy, V.R. Edgerton, Differential regulation by exercise of BDNF and NT-3 in rat spinal cord and skeletal muscle, Eur. J. Neurosci. 13 (2001) 1078–1084.

[33] M.B. Graeber, E. Grasbon-Frodl, P. Abell-Aleff, S. Kosel, Nigral neurons are likely to die of a mechanism other than classical apoptosis in Parkinson's disease., Parkinsonism Relat. Disord. 5 (1999) 187–192.

[34] A.C. Granholm, M. Reyland, D. Albeck, L. Sanders, G. Gerhardt, G. Hoernig, L. Shen, H. Westphal, B. Hoffer, Glial cell line-derived neurotrophic factor is essential for postnatal survival of midbrain dopamine neurons, J. Neurosci. 20 (2000) 3182–3190.

[35] E.J. Green, W.T. Greenough, B.E. Schlumpf, Effects of complex or isolated environments on cortical dendrites of middle-aged rats, Brain Res. 264 (1983) 233–240.

[36] W.T. Greenough, F.R. Volkmar, J.M. Juraska, Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat, Exp. Neurol. 41 (1973) 371–378.

[37] R. Grondin, Z. Zhang, A. Yi, W.A. Cass, N. Maswood, A.H. Andersen, D.D. Elsberry, M.C. Klein, G.A. Gerhardt, D.M. Gash, Controlled GDNF infusion promotes structural and functional recovery in advanced parkinsonian monkeys, Brain 125 (2002) 2191–2201.

[38] K. Hamre, R. Tharp, K. Poon, X. Xiong, R.J. Smeyne, Differential strain susceptibility following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration acts in an autosomal dominant fashion: quantitative analysis in seven strains of *Mus musculus*, Brain Res. 828 (1999) 91–103.

[39] S. Heck, F. Lezoualc'h, S. Engert, C. Behl, Insulin-like growth factor-1-mediated neuroprotection against oxidative stress is associated with activation of nuclear factor kappaB, J. Biol. Chem. 274 (1999) 9828–9835.

[40] B.R. Ickes, T.M. Pham, L.A. Sanders, D.S. Albeck, A.H. Mohammed, A.C. Granholm, Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain, Exp. Neurol. 164 (2000) 45–52.

[41] V. Jackson-Lewis, M. Jakowec, R.E. Burke, S. Przedborski, Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Neurodegeneration 4 (1995) 257–269.

[42] P. Jenner, C.D. Marsden, The actions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in animals as a model of Parkinson's disease., J. Neural Transm., Suppl. 20 (1986) 11–39.

[43] G. Kempermann, H.G. Kuhn, F.H. Gage, Experience-induced neurogenesis in the senescent dentate gyrus, J. Neurosci. 18 (1998) 3206–3212.

[44] I.J. Kopin, Features of the dopaminergic neurotoxin MPTP, Ann. N.Y. Acad. Sci. 648 (1992) 96–104.

[45] D. Krech, M.R. Rosenzweig, E.L. Bennett, Relations between brain chemistry and problem-solving among rats raised in enriched and impoverished environments, J. Comp. Physiol. Psychol. 55 (1962) 801.

[46] J.W. Langston, P. Ballard, J.W. Tetrud, I. Irwin, Chromic Parkinsonism in humans due to a product of merperidine-analog synthesis., Science 219 (1983) 979–980.

[47] I. Lerman, B.C. Harrison, K. Freeman, T.E. Hewett, D.L. Allen, J. Robbins, L.A. Leinwand, Genetic variability in forced and voluntary endurance exercise performance in seven inbred mouse strains, J. Appl. Physiol. 92 (2002) 2245–2255.
[48] M. Mabandla, L. Kellaway, A. St Clair Gibson, V.A. Russell, Voluntary running provides neuroprotection in rats after 6-hydroxydopamine injection into the medial forebrain bundle, Metab. Brain Dis. 19 (2004) 43–50.
[49] J.L. McBride, J.H. Kordower, Neuroprotection for Parkinson's disease using viral vector-mediated delivery of GDNF, Prog. Brain Res. 138 (2002) 421–432.
[50] G.W. Miller, R.R. Gainetdinov, A.I. Levey, M.G. Caron, Dopamine transporters and neuronal injury, TIPS 20 (1999) 424–429.
[51] Y. Mizuno, N. Sone, K. Suzuki, T. Saitoh, Studies on the toxicity of 1-methyl-4-phenylpyridinium ion (MPP^+) against mitochondria of mouse brain, J. Neurol. Sci. 86 (1988) 97–110.
[52] G. Moretto, R.Y. Xu, D.G. Walker, S.U. Kim, Co-expression of mRNA for neurotrophic factors in human neurons and glial cells in culture, J. Neuropathol. Exp. Neurol. 53 (1994) 78–85.
[53] S.A. Neeper, F. Gomez-Pinilla, J. Choi, C. Cotman, Exercise and brain neurotrophins, Nature 373 (1995) 109.
[54] S.A. Neeper, F. Gomez-Pinilla, J. Choi, C.W. Cotman, Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain, Brain Res. 726 (1996) 49–56.
[55] W.J. Nicklas, S.K. Youngster, M.V. Kindt, R.E. Heikkila, MPTP, MPP^+ and mitochondrial function, Life Sci. 40 (1987) 721–729.
[56] J.G. Nutt, K.J. Burchiel, C.L. Comella, J. Jankovic, A.E. Lang, E.R. Laws Jr., A.M. Lozano, R.D. Penn, R.K. Simpson Jr., M. Stacy, G.F. Wooten, Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD, Neurology 60 (2003) 69–73.
[57] T. Obata, Role of hydroxyl radical formation in neurotoxicity as revealed by in vivo free radical trapping, Toxicol. Lett. 132 (2002) 83–93.
[58] J.A. Obeso, M.C. Rodriguez-Oroz, M. Rodriguez, R. Macias, L. Alvarez, J. Guridi, J. Vitek, M.R. DeLong, Pathophysiologic basis of surgery for Parkinson's disease, Neurology 55 (2000) S7–S12.
[59] H.S. Oliff, N.C. Berchtold, P. Isackson, C.W. Cotman, Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus, Mol. Brain Res. 61 (1998) 147–153.
[60] P. Palanza, Animal models of anxiety and depression: how are females different? Neurosci. Biobehav. Rev. 25 (2001) 219–233.
[61] G. Paxinos, K.B.J. Franklin, The Mouse Brain in Stereotaxic Coordinates, Academic Press, San Diego, 2001.
[62] F. Piccoli, V. La Bella, R. Guarneri, New insights on the pathogenesis of neurodegenerative disorders, Acta Neurol. (Napoli) 14 (1992) 455–468.
[63] I. Reuter, M. Engelhardt, K. Stecker, H. Baas, Therapeutic value of exercise training in Parkinson's disease, Med. Sci. Sports Exerc. 31 (1999) 1544–1549.
[64] H. Rollema, W.G. Kuhr, G. Kranenborg, J. De Vries, C. Van den Berg, $MPP\pm$induced efflux of dopamine and lactate from rat striatum have similar time courses as shown by in vivo brain dialysis, JPET 245 (1988) 858–866.
[65] C. Rosenblad, D. Kirik, B. Devaux, B. Moffat, H.S. Phillips, A. Bjorklund, Protection and regeneration of nigral dopaminergic neurons by neurturin or GDNF in a partial lesion model of Parkinson's disease after administration into the striatum or the lateral ventricle, Eur. J. Neurosci. 11 (1999) 1554–1566.
[66] A.A. Russo-Neustadt, R.C. Beard, Y.M. Huang, C.W. Cotman, Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus, Neuroscience 101 (2000) 305–312.
[67] T. Saitoh, K. Niijima, Y. Mizuno, Long-term effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on striatal dopamine content in young and mature mice, J. Neurol. Sci. 77 (1987) 229–235.
[68] J. Schmidt, K. Mertz, J.I. Morgan, Regulation of heme oxygenase-1 expression by dopamine in cultured C6 glioma and primary astrocytes, Mol. Brain Res. 73 (1999) 50–59.
[69] M. Smeyne, O. Goloubeva, R.J. Smeyne, Strain-dependent susceptibility to MPTP and MPP^+-induced Parkinsonism is determined by glia, Glia 74 (2001) 73–80.
[70] M. Smeyne, R.J. Smeyne, Method for culturing postnatal substantia nigra as an in vitro model of experimental Parkinson's disease, Brain Res. Protoc. 9 (2002) 105–111.
[71] A.D. Smith, M.J. Zigmond, Can the brain be protected through exercise? Lessons from an animal model of parkinsonism, Exp. Neurol. 184 (2003) 31–39.
[72] P.S. Spencer, A.C. Ludolph, G.E. Kisby, Are human neurodegenerative disorders linked to environmental chemicals with excitotoxic properties? Ann. N.Y. Acad. Sci. 648 (1992) 154–160.
[73] R.G.W. Staal, P.K. Sonsalla, Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata, JPET 293 (2000) 336–342.
[74] M. Takada, M. Matsumura, J. Kojima, Y. Yamaji, M. Inase, H. Tokuno, A. Nambu, H. Imai, Protection against dopaminergic nigrostriatal cell death by excitatory input ablation, Eur. J. Neurosci. 12 (2000) 1771–1780.
[75] C.M. Tanner, D.A. Aston, Epidemiology of Parkinson's disease and akinetic syndromes, Curr. Opin. Neurol. 13 (2000) 427–430.
[76] N.A. Tatton, S.J. Kish, In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labelling and acridine orange staining, Neuroscience 77 (1997) 1037–1048.
[77] J.L. Tillerson, W.M. Caudle, M.E. Reveron, G.W. Miller, Exercise induces behavioral recovery and attenuates neurochemical deficits in rodent models of Parkinson's disease, Neuroscience 119 (2003) 899–911.
[78] J.L. Tillerson, A.D. Cohen, J. Philhower, G.W. Miller, M.J. Zigmond, T. Schallert, Forced limb-use effects on the behavioral and neurochemical effects of 6-hydroxydopamine, J. Neurosci. 21 (2001) 4427–4435.
[79] T. Tsukahara, M. Takeda, S. Shimohama, O. Ohara, N. Hashimoto, Effects of brain-derived neurotrophic factor on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in monkeys, Neurosurgery 37 (1995) 733–739 discussion 739-41.
[80] A.M. Turner, W.T. Greenough, Differential rearing effects on rat visual cortex synapses: I. Synaptic and neuronal density and synapses per neuron, Brain Res. 329 (1985) 195–203.
[81] H. van Praag, G. Kempermann, F.H. Gage, Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus, Nat. Neurosci. 2 (1999) 266–270.
[82] G.A. Werther, M. Abate, A. Hogg, H. Cheesman, B. Oldfield, D. Hards, P. Hudson, B. Power, K. Freed, A.C. Herington, Localization of insulin-like growth factor-I mRNA in rat brain by in situ hybridization-relationship to IGF-I receptors, Mol. Endocrinol. 4 (1990) 773–778.
[83] L.R. Williams, Oxidative stress, age-related neurodegeneration, and the potential for neurotrophic treatment, Cerebrovasc. Brain Metab. Rev. 7 (1995) 55–73.
[84] D. Young, P.A. Lawlor, P. Leone, M. Dragunow, M.J. During, Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective, Nat. Med. 5 (1999) 448–453.