Neuroscience Letters 450 (2009) 102–105

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Effects of endurance exercise on ventral tegmental area neurons in the chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and probenecid-treated mice

S. Omar Ahmad[a,*], Ji-Hyuk Park[a], Lisa Stenho-Bittel[b], Yuen-Sum Lau[c,d]

[a] Department of Occupational Therapy Education, University of Kansas Medical Center, Mail Stop 2003, 3901 Rainbow Boulevard, Kansas City, KS 66160, United States
[b] Department of Physical Therapy and Rehabilitation Science, University of Kansas Medical Center, Kansas City, KS, United States
[c] Division of Pharmacology, University of Missouri-Kansas City, Kansas City, MO 64108, United States
[d] Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX 77584, United States

ARTICLE INFO

Article history:
Received 15 August 2008
Received in revised form 11 November 2008
Accepted 29 November 2008

Keywords:
A10 neurons
Chronic mouse model
Parkinson's disease
Rodent treadmill
Stereology
TH immunoreactivity

ABSTRACT

Loss of dopaminergic neurons in the substantia nigra (A9 cells) and ventral tegmental area (VTA) (A10 cells) has been reported in Parkinson's disease with reference to causing motor and non-motor deficits, although clinical and laboratory animal studies on the degeneration of VTA neurons are less emphasized comparative to the degeneration of substantia nigra neurons. In the present study, we examined the VTA dopaminergic neurons in a chronic mouse model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and probenecid at a level showing moderate neurodegeneration and studied the impact of endurance exercise on VTA neurons in this model. In comparison to the normal control animals, the chronic mouse model of Parkinson's disease with moderate neurodegeneration demonstrated a significant reduction of VTA neurons (52% loss), when these animals were kept sedentary throughout the study. Morphologically, the VTA dopaminergic neurons in this model displayed a decrease in cell volume and showed irregular or disparaging axonal and dendritic projections. When the chronic Parkinsonian mice were exercised on a motorized rodent treadmill up to 15 m/min, 40 min/day, 5 days/week for 10 and 18 weeks, the total number of VTA dopaminergic neurons were significantly higher than the sedentary Parkinsonian animals. Especially noted with the 18-week exercised Parkinsonian mice, the number of VTA neurons returned to normal range and the cells were densely populated and displayed distinctive axons and dendritic arborization. These results demonstrate that prolonged exercise training is neuroprotective to the dopaminergic neurons in the VTA of the chronic mouse model of Parkinson's disease with moderate neurodegeneration.

Parkinson's disease (PD) is a known neurodegenerative disorder afflicting millions of people in the growing aging population. Patients with PD are debilitated with symptoms of muscular rigidity, impaired movement, loss of balance, cognitive disabilities and tremor at rest. So far, both clinical and experimental research have convincingly determined that the underlying pathology of PD is involved in the loss of A9 cells in the substantia nigra pars compacta leading to nigrostriatal neuron degeneration and depletion of the transmitter, dopamine (DA). Although it is less emphasized, examination of the postmortem PD brain specimens has further revealed a loss of A10 dopaminergic cells in the ventral tegmental area (VTA) adjacent to the substantia nigra [2,11]. In acute and subacute animal models of Parkinsonism induced by the dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), although early studies did not detect neuronal loss in the A10 VTA region [10], subsequent investigations have confirmed that MPTP causes a significant loss of the A10 VTA neurons in addition to the A9 substantia nigra neurons in various species of animals including the mouse [3,5].

The cell bodies of DA neurons that originate from the VTA, project to, and terminate at several parts of the brain have demonstrated functional significance. In general, the dopaminergic connection between the VTA and nucleus accumbens (the mesolimbic system) has been linked to reward and reinforcement behaviors [4]. The VTA dopaminergic neurons that project to the cerebral cortex, most heavily to the prefrontal lobe (the mesocortical system) are believed to be involved in maintaining cognitive functions [16] and in controlling stress and emotional responses [18]. Thus, cell loss or disruption of DA transmission in the VTA neurons associated with PD may lead to non-motor type of behavioral dysfunction.

Clinical studies have shown that exercise tends to improve motor performance and ambulation in PD patients [12]. In the acute MPTP-induced mouse model of Parkinsonism, treadmill exercise is shown to ameliorate behavioral and dopaminergic deficits [19]. However,

* Corresponding author. Tel.: +1 913 588 7195.
E-mail address: sahmad1@kumc.edu (S.O. Ahmad).

0304-3940/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2008.11.065

in the chronic MPTP/probenecid-induced mouse model of Parkinsonism (MPD) with severe dopaminergic neurodegeneration, we have demonstrated that 4 weeks of treadmill exercise did not lead to neuronal recovery, even though the animals showed significant cardiovascular and metabolic rehabilitation [1]. In the present study, we examined the effects of exercise on VTA dopaminergic cells in the chronic MPD with moderate dopaminergic neurodegeneration by using the unbiased, design-based stereology.

Ten to twelve week-old, male, C57BL/6 mice (Charles River Laboratories, Inc., Wilmington, MA, USA), weighing between 25 and 27 g at the beginning of the study were housed in single cages with food pellets and water available *ad libitum*. The room was maintained at a constant temperature and humidity on a 12-h/12-h light/dark cycle. A total of 30 mice were used in the present study. All animal treatments were carried out strictly according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised 1996) and were approved by the Institutional Animal Care and Use Committees from the University of Missouri-Kansas City. This investigation was conducted with assurance that a minimal number of animals were used and the experimental procedures did not significantly cause animal suffering.

To prepare the chronic MPD with moderate neurodegeneration, mice were injected with a total of 10 doses of MPTP hydrochloride (12.5 mg/kg in saline, s.c.) in combination with an adjuvant, probenecid (250 mg/kg in dimethyl sulfoxide, DMSO, i.p.). The 10 doses were administered on a 5-week schedule with an interval of 3.5 days between consecutive doses. Probenecid is known to promote MPTP accumulation in the brain and to potentiate its neurotoxic effect by impeding the renal excretion and neuronal clearance of MPTP and its toxic metabolites [9]. MPTP hydrochloride and probenecid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Safety precautions for the use of MPTP during chemical preparation and animal injections were according to the procedures previously described [8]. Control animals were treated with saline or the same dose of probenecid. Probenecid used in this study does not produce a significant level of degeneration to the dopaminergic neurons [15].

A six-lane motorized rodent treadmill (Columbus Instruments, Columbus, OH, USA) was utilized for exercise training. One week before, 5 weeks during, and 4 or 12 weeks after the chronic MPTP/Probenecid treatment, the exercised group of animals (for a total of 10 or 18 weeks of exercise) was trained on the treadmill running for 5 days/week, 40 min/day at a speed up to 15 m/min (5 min at 6 m/min, 5 min at 9 m/min, 20 min at 12 m/min, 5 min at 15 m/min, and 5 min at 12 m/min) with 0° of inclination. Using this treadmill exercise protocol in our laboratory, the chronic MPD mice were able to go through the training with minimal requirement for external stimuli or manual prodding, yet the animals developed physical endurance after 4 weeks showing cardiorespiratory and metabolic adaptations comparable to those seen in human subjects undergoing continuous exercise training [1]. Sedentary mice did not exercise; however, they were transported daily to the training room so that they were exposed to the same environment as the exercised group of animals.

At the specified ending period of exercise training, mice were anesthetized with pentobarbital (130 mg/kg; Sigma), transcardially perfused with 40–60 ml of 4% sucrose in 0.1 M phosphate-buffered saline (PBS), followed by 70 ml of 4% paraformaldehyde and 4% sucrose in PBS. The brains were removed, placed in the perfusion fixative for 4 h at room temperature and then transferred to PBS and kept at 4 °C. The brain samples were embedded with the MultiBrain™ technology, sectioned coronally and immunostained with tyrosine hydroxylase (TH) (NeuroScience Associates, Knoxville, TN). This method of preparation yielded minimal shrinkage (slices were cut at 50 μm and reduced to 30.87 μm) and no evidence of tissue distortion. Every fourth section of the VTA was collected and 6–8 sections per animal were analyzed unilaterally. In order to estimate changes in the number of TH+ cells in VTA, we implemented the modern unbiased, design-based stereology as previously described [17]. The slides were checked precisely against an anatomical atlas when defining the reference space [14]. Examples of parsing the VTA are provided in Fig. 1A–C. The VTA is located in the ventral tegmentum of the mesencephalon. Anteriorly, it is bordered by the lateral hypothalamic area. Dorsomedially, it is bordered by the substantia nigra, pars compacta and reticulate.

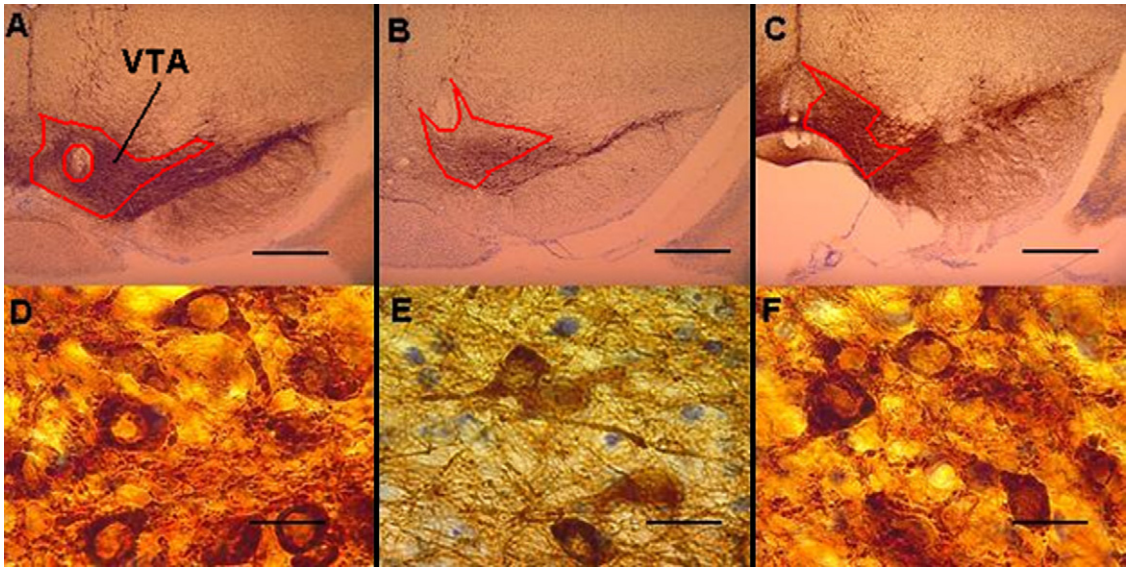
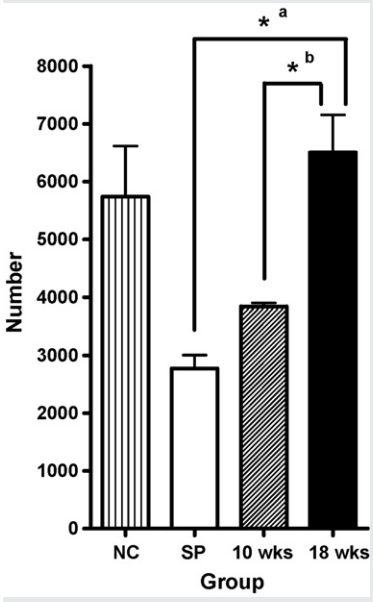
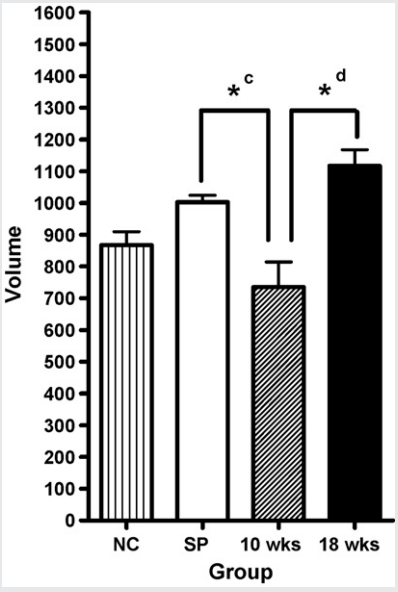


Fig. 1. Representative light microscope photomicrographs illustrating TH+ immunoreactivity under low power (144X, panels A–C) and morphological structures of the VTA neurons under high power (3600×, panels D–F) in control (A, D), sedentary chronic MPD (B, E), and 18-week exercise-trained chronic MPD (C, F). It is noticeable that the density of VTA TH+ immunoreactivity in sedentary chronic MPD (B) was significantly reduced when compared to the control animal (A). VTA TH+ immunoreactivity returned to normal in chronic MPD following 18 weeks of exercise training (C). Morphological examinations at a higher magnification revealed that in association with a sparse neuronal number, the TH+ cells had a reduced volume and depleted dendritic extensions in the sedentary chronic MPD (E) comparing to the control animal (D). Exercise training for 18 weeks in the chronic MPD led to a prominent recovery of the VTA TH+ neurons demonstrating neuronal clustering and extensive dendritic arborization (F) as similarly detected in the normal control animal (D).

Table 1
Effects of endurance exercise training on the total number (*N*) and volume (*V*) of VTA TH+ neurons in the chronic MPD.

Number of neuron	Volume of neuron
	

TH+ neurons in the VTA of normal controls, sedentary chronic MPD, 10- and 18-week exercise-trained chronic MPD were counted by a random and unbiased stereological approach and the data were compared. [a]Significantly lower than the control mice, $p = 0.002$. [b]Significantly lower than the control mice, $p = 0.001$ and significantly higher than the sedentary chronic MPD, $p = 0.01$. [c]Significantly higher than the sedentary chronic MPD and the 10-week exercise-trained chronic MPD, $p < 0.05$, and not significantly different from the control mice. Mean cell body volume was significant exercised MPD and sedentary MPD, $p = .004$, and [d]exercised chronic MPD 10 weeks and exercised MPD 18 weeks, $p = 000$.

Laterally, it is bordered by the medial lemniscus and medially by the caudo-linear nucleus of Raphae. Posteriorly, it is bordered by the deep mesencephalic nucleus [14]. Only those cells demonstrating clear visualization of the nucleolus and precise definition of the cell membrane were counted.

After mice were treated chronically with MPTP/probenecid and remained sedentary for additional 4 or 12 weeks, we detected a 55% loss of TH+ cells in the SNpc confirming the nigral neurodegeneration in the moderate chronic MPD. Interestingly, a significant 52% loss of TH+ cells was also detected in the VTA (Table 1). The loss of VTA TH+ neurons in the sedentary chronic MPD between 4 and 12 weeks post-treatment was not significantly different; therefore, the results from these two groups of animals are presented as combined. Continuous treadmill exercise training for 4 weeks after the completion of chronic MPTP/probenecid treatment in mice (total of 10 weeks of exercise), there was a small but statistically significant increase in the number of VTA TH+ cells comparing to that of the sedentary chronic MPD (Table 1). Following 18 weeks of exercise training (or 12 weeks after treatment) in the chronic MPD, the number of TH+ cells in VTA was not only significantly greater than that of the sedentary chronic MPD, the number even returned to the normal level as seen in the non-Parkinsonian control mice (Table 1).

The morphological characteristics of VTA TH+ neurons in control, sedentary chronic MPD, 10- and 18-week exercised chronic MPD were investigated and compared. The neuroprotective effect of exercise in 18-week exercised chronic MPD was especially noteworthy, which is illustrated in this report. Microscopic examination of TH+ neurons in the VTA under low power revealed that there was a substantial loss of dopaminergic immunoactivity 12 weeks after chronic MPD treatment (or 18 weeks from the beginning of the study) in the sedentary animals (Fig. 1, panel B) when compared with the control animals (Fig. 1, panel A). Treadmill exercise for 18 weeks in chronic MPD prevented the loss of dopaminergic immunoactivity (Fig. 1, panel C) when compared with the sedentary MPD mice (Fig. 1, panel B). In addition to the quantitative loss of VTA TH+ neurons in the sedentary chronic MPD (Table 1), significant morphological changes in these neurons were also detected under high power microscopic magnification. The sedentary chronic MPD displayed sparsely distributed TH+ neurons with reduced cell volume plus irregular or disparaging axonal and dendritic projections (Fig. 1, panel E). On the other hand, the morphological features of TH+ neurons in 18-week exercise-trained chronic MPD presented a dense neuronal network consisting of well-defined cells with distinctive axons and dendritic arborization (Fig. 1, panel F) similar to those found in the control animals (Fig. 1, panel D). Quantitatively, mean cell body volume reflected these changes, which were quantified using an isotropic random uniform stererological procedure (Table 1). Taken these findings together, our study demonstrates that prolonged exercise training exhibits a neuroprotective effect to the dopaminergic neurons in the VTA of the chronic MPD. We are also currently conducting our studies investigating the impact of exercise to the nigrostriatal neurons in the chronic MPD.

The chronic MPD that is induced by MPTP/probenecid and used in this study affords a neurodegenerative model for PD. With a high dose of MPTP (25 mg/kg), the chronic MPD attains a severe level of neurodegeneration showing neurochemical, histological, behavioral and pathological characteristics resembling that in PD at advanced stages [6,7,13]. Administering exercise to the severe chronic MPD does not appear to produce neuroprotective or neuroregenerative effects to the dopaminergic neurons [1]. We do not expect that exercise or other pharmacotherapeutic approaches would offer such promises in the severe chronic MPD or advanced PD, in which a majority of neurons and neurotransmitters are already permanently lost. However, using a mild to moderate PD model as demonstrated in the present study, it is encouraging to find that continuous exercise produces neuroprotection and deters neurotoxic and neurodegenerative action of MPTP. If the laboratory animal results can be translated to clinical predictions, our findings suggest that maintaining endurance exercise in the early stage of neurodegenerative disorders may protect against or slow down the

progression of neurodegeneration. The underlying mechanisms of exercise on neuroprotection is unknown and whether exercise has any neurorestorative potential after neurodegenerative process has already initiated or established require further investigations.

Acknowledgments

The authors thank Dr. L. Novikova for her assistance in exercise training of the animals and in sample collection. This study was supported by a grant from the National Institute of Neurological Disorders and Stroke to YSL (NS 47920).

References

[1] M. Al-Jarrah, K. Pothakos, L. Novikova, I.V. Smirnova, M.J. Kurz, L. Stehno-Bittel, Y.S. Lau, Endurance exercise promotes cardiorespiratory rehabilitation without neurorestoration in the chronic mouse model of Parkinsonism with severe neurodegeneration, Neuroscience 149 (2007) 28–37.
[2] D.C. German, K. Manaye, W.K. Smith, D.J. Woodward, C.B. Saper, Midbrain dopaminergic cell loss in Parkinson's disease: computer visualization, Ann. Neurol. 26 (1989) 507–514.
[3] D.C. German, E.L. Nelson, C.L. Liang, S.G. Speciale, C.M. Sinton, P.K. Sonsalla, The neurotoxin MPTP causes degeneration of specific nucleus A8, A9 and A10 dopaminergic neurons in the mouse, Neurodegeneration 5 (1996) 299–312.
[4] S. Ikemoto, J. Panksepp, The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking, Brain Res. Rev. 31 (1999) 6–41.
[5] 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.
[6] M.J. Kurz, K. Pothakos, S. Jamaluddin, M. Scott-Pandorf, C. Arellano, Y.S. Lau, A chronic mouse model of Parkinson's disease has a reduced gait pattern certainty, Neurosci. Lett. 429 (2007) 39–42.
[7] Y.S. Lau, Progressive neurodegeneration in the chronic MPTP/probenecid model of Parkinson's disease, in: M. Ebadi, R. Pfeiffer (Eds.), Parkinson's Disease, CRC Press, Boca Raton, FL, 2005, pp. 109–115.
[8] Y.S. Lau, L. Novikova, C. Roels, MPTP treatment in mice does not transmit and cause Parkinsonian neurotoxicity in non-treated cagemates through close contact, Neurosci. Res. 52 (2005) 371–378.
[9] Y.S. Lau, K.L. Trobough, J.M. Crampton, J.A. Wilson, Effects of probenecid on striatal dopamine depletion in acute and long-term 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice, Gen. Pharmacol. 21 (1990) 181–187.
[10] S.P. Markey, J.N. Johannessen, C.C. Chiueh, R.S. Burns, M.A. Herkenham, Intraneuronal generation of a pyridinium metabolite may cause drug-induced Parkinsonism, Nature 311 (1984) 464–467.
[11] D.A. McRitchie, H.R. Cartwright, G.M. Halliday, Specific A10 dopaminergic nuclei in the midbrain degenerate in Parkinson's disease, Exp. Neurol. 144 (1997) 202–213.
[12] I. Miyai, Y. Fujimoto, Y. Ueda, H. Yamamoto, S. Nozaki, T. Saito, J. Kang, Treadmill training with body weight support: its effect on Parkinson's disease, Arch. Phys. Med. Rehabil. 81 (2000) 849–852.
[13] L. Novikova, B.L. Garris, D.R. Garris, Y.S. Lau, Early signs of neuronal apoptosis in the substantia nigra pars compacta of the progressive neurodegenerative mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid model of Parkinson's disease, Neuroscience 140 (2006) 67–76.
[14] G. Paxinos, K. Franklin, Themouse Brain in Stereotaxic Coordinates, second ed., Academic Press, New York, NY, 2001.
[15] E. Petroske, G.E. Meredith, S. Callen, S. Totterdell, Y.S. Lau, Mouse model of Parkinsonism: a comparison between subacute MPTP and chronic MPTP/probenecid treatment, Neuroscience 106 (2001) 589–601.
[16] A.G. Phillips, S. Ahn, S.B. Floresco, Magnitude of dopamine release in medial prefrontal cortex predicts accuracy of memory on a delayed response task, J. Neurosci. 24 (2004) 547–553.
[17] S. Scott, N. Diaz, S. Ahmad, Stereologic analysis of cell number and size during postnatal development in the rat substantia nigra, Neurosci. Lett. 419 (2007) 34–37.
[18] R.M. Sullivan, Hemispheric asymmetry in stress processing in rat prefrontal cortex and the role of mesocortical dopamine, Stress 7 (2004) 131–143.
[19] 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.