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Treadmill exercise reverses dendritic spine loss in direct and indirect striatal medium spiny neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease☆

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ABSTRACT

Exercise has been shown to be beneficial for Parkinson's disease (PD). A major interest in our lab has been to investigate how exercise modulates basal ganglia function and modifies disease progression. Dopamine (DA) depletion leads to loss of dendritic spines within the caudate nucleus and putamen (striatum) in PD and its animal models and contributes to motor impairments. Striatal medium spiny neurons (MSNs) can be delineated into two populations, the dopamine D_1 receptor (DA-D_1R)-containing MSNs of the direct pathway and dopamine D_2 receptor (DA-D_2R)-containing MSNs of the indirect pathway. There is evidence to suggest that the DA-D_2R-indirect pathway MSNs may be preferentially affected after DA-depletion with a predominate loss of dendritic spine density when compared to MSNs of the DA-D_1R-direct pathway in rodents; however, others have reported that both pathways may be affected in primates. The purpose of this study was to investigate the effects of intensive exercise on dendritic spine density and arborization in MSNs of these two pathways in the MPTP mouse model of PD. We found that MPTP led to a decrease in dendritic spine density in both DA-D_1R- and DA-D_2R-containing MSNs and 30 days of intensive treadmill exercise led to increased dendritic spine density and arborization in MSNs of both pathways. In addition, exercise increased the expression of synaptic proteins PSD-95 and synaptophysin. Taken together these findings support the potential effect of exercise in modifying synaptic connectivity within the DA-depleted striatum and in modifying disease progression in individuals with PD.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of midbrain dopaminergic neurons leading to the depletion of striatal dopamine (DA). Individuals with PD manifest both cognitive and motor deficits including balance instability, gait dysfunction, slowness, tremor, and rigidity. While DA replacement therapy remains a cornerstone of treatment, its efficacy diminishes over time. Currently, there is no known cure for PD. A major interest of research in PD is to find treatments that have the capacity to modify disease progression or better prevent disease onset. For example, epidemiological studies have suggested that intensive exercise, especially in males, over a lifetime can influence the occurrence of PD (Chen et al., 2005). Over the last decade a number of investigators have demonstrated exercise to be beneficial for the treatment of PD especially for the treatment of gait and balance (Petzinger et al., 2010, 2013; Speelman et al., 2011). In animal models of PD, exercise has been shown to have the capacity to be both neuroprotective, by preventing the loss of DA in lesion models (Gerecke et al., 2010), and neurorestorative, providing enhancement in DA neurotransmission leading to reversal of motor deficits (Fisher et al., 2004; Petzinger et al., 2007). These observations, along with ongoing basic and clinical research, are beginning to investigate the potential role of exercise in modifying disease progression in individuals with PD as well as in animal models of DA-depletion. Towards this goal, a major focus of our lab has been to elucidate the underlying molecular mechanisms by which exercise affects

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neuroplasticity including synaptic structure and function within the injured basal ganglia in neurotoxin animal models of PD.

In PD and in neurotoxin animal models, the depletion of striatal DA leads to alterations in basal ganglia neurotransmission that manifest in a number of different ways. For example, the striatopallidal (indirect) projection medium spiny neurons (MSNs) become hyperactive and are thought to underlie the onset of akinesia (slowness of movement) (Calabresi et al., 1997). In addition, corticostriatal projections targeting striatal MSNs also display aberrant glutamatergic neurotransmission as demonstrated through electrophysiological techniques (Cepeda et al., 1989; Pisani et al., 2005). Therefore, it is not surprising that striatal MSNs manifest these changes in neurotransmission by alterations in the dendritic spine number and dendritic arborization, the primary morphological correlates of synaptic neurotransmission. For example, studies using Golgi staining have shown a reduction in dendritic arborization and spine density in the caudate nucleus and putamen in tissues from patients with PD (McNeill et al., 1988; Stephens et al., 2005). Similar findings have been made in both the 6-hydroxydopamine (6-OHDA) rat and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) nonhuman primate models of DA-depletion where spine density changes have been reported (Ingham et al., 1989, 1993; Villalba and Smith, 2010).

The purpose of this study was to determine if intensive exercise in the form of treadmill running leads to a reversal of both dendritic spine loss and the reduction in dendritic arborization in a mouse model of DA-depletion. This speculation is largely based on the fact that we observe exercise enhanced alterations in both glutamate and DA neurotransmission in the MPTP-lesioned mouse model (Kintz et al., 2013). Since dendritic spine density and branching are influenced by experience including environmental enrichment and exercise in several regions of the mammalian brain we were interested in knowing if exercise leads to a similar outcome (Comery et al., 1995; Eadie et al., 2005; Leggio et al., 2005; Stranahan et al., 2007). We also took advantage of the Drd_2-eGFP-BAC transgenic mouse strain (Chan et al., 2012; Nelson et al., 2012) in conjunction with biocytin injection methods that allowed us to delineate dopamine D_1 receptor (DA-D_1R)-containing MSNs and dopamine D_2 receptor (DA-D_2R)-containing MSNs since some models of DA-depletion show preferential loss of dendritic spine density selectively on DA-D_2R-containing MSNs (Day et al., 2006). Utilizing the MPTP mouse model of DA-depletion with either Golgi staining or biocytin labeling we examined the effects of 6 weeks of intensive treadmill running on dendritic spine density and arborization of MSNs within the dorsolateral striatum, a region responsible for motor control and a site where we have documented neuroplastic changes in response to exercise (Petzinger et al., 2007).

Material and methods

Animals

Twelve C57BL/6J young adult (8 to 10 weeks old) male mice from Jackson Labs (Bar Harbor, Maine) and 52 young adult (8 to 10 weeks old) male Drd_2-eGFP-BAC mice (Tg(Drd2-EGFP)118Gsat/Mmnc) supplied by the Mutant Mouse Regional Resource Center of NIH (MMRRC) program at the University of California, Davis (Gong et al., 2003) were used for this study. We have established a colony of Drd_2-eGFP-BAC mice that have been backcrossed into C57BL/6J mice in our lab at least 10 times to enhance genomic stability and validate comparison of outcomes with those derived from C57BL/6J mice. Male hemizygous mice Drd_2-eGFP-BAC mice continually backcrossed onto C57BL/6J mice were used for this study. Both groups of C57BL/6J and Drd_2-eGFP-BAC mice were randomly assigned to one of four treatment groups including: (i) saline, (ii) saline + exercise, (iii) MPTP, and (iv) MPTP + exercise. Mice were group housed with a reverse light cycle (lights off 7 a.m. to 7 p.m.) with ad libitum access to food and water. Experimental procedures were approved by the University of Southern California's Institutional Animal Care and Use Committee and conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (DHEW Publication 80-23, 2011, Office of Laboratory Animal Welfare, DRR/NIH, Bethesda, MD). All efforts were made to minimize animal suffering and the number of animals used to achieve statistical significance.

Some concern has been raised about the Drd_2-eGFP-BAC mouse line and the possibility that this BAC line does not express the physiology and associated behavior seen in C57BL/6J mice (Kramer et al., 2011). However, several reports indicate that Drd_2-eGFP-BAC mice back crossed to C57BL/6J mice display both normal behavior and DA neurotransmission (Chan et al., 2012; Nelson et al., 2012; Taverna et al., 2008). In our hands no differences were detectable between Drd_2-eGFP-BAC mice and C57BL/6J mice in maximum running velocity on the treadmill, normal striatal DA-levels, amount of DA-depletion, or degree of substantia nigra pars compacta dopaminergic cell death resulting from systemic injections of MPTP in our striatal lesioning protocol (Jackson-Lewis et al., 1995; Kintz et al., 2013).

MPTP-lesioning

Half of the C57BL/6J and half of the Drd_2-eGFP-BAC mice were administered 4 intraperitoneal injections of MPTP at 20 mg/kg (free-base, Sigma, St. Louis, MO) at 2-hour intervals. Remaining mice received intraperitoneal injections of saline. This lesioning regimen results in 90–95% loss of striatal dopamine and 65 to 70% loss of nigrostriatal dopaminergic neurons (Jackson-Lewis et al., 1995; Jakowec et al., 2004). Drd_2-eGFP-BAC mice on the C57BL/6J background had DA-depletion and nigrostriatal cell death indistinguishable from wild-type C57BL/6J mice (Kintz et al., 2013). In this study, striatal DA levels of Drd_2-eGFP-BAC mice were assessed by high-performance liquid chromatography (HPLC) analysis. HPLC analysis was conducted 5 days after lesioning, corresponding to the start of the treadmill exercise regimen, and at 42 days after lesioning, corresponding to the completion of the treadmill exercise regimen.

Exercise regimen

1 week before the start of the treadmill exercise regimen (2 days before MPTP lesioning), 8 to 10 week old C57BL/6J and Drd_2-eGFP-BAC mice that could maintain a forward position on the 45 cm treadmill belt for 5 min at 5.0 m/min were randomly assigned to the 4 groups to ensure that all animals performed similarly on the treadmill task prior to MPTP-lesioning. The treadmill used in these studies was a Model EXER-6M Treadmill manufactured by Columbus Instruments (Columbus, Ohio). A non-noxious stimulus (metal beaded curtain) was used as a tactile incentive to prevent animals from drifting back on the treadmill. The treadmill exercise regimen was conducted as previously described (Fisher et al., 2004). Briefly, exercise was initiated 5 days following MPTP or saline administration, a time point after cell death is complete, and continued 5 days/week for a total of 6 weeks of exercise (corresponding to a total of 42 days after MPTP-lesioning). Exercised mice started at a velocity of 10.0 m/min, which increased to 24.0 m/min by the final week. These velocities were adjusted (either increased or decreased) such that all mice within a group could maintain a forward position on the treadmill for 75% of the running period. At the end of each week, the average achieved velocities were calculated to produce a time course of improvement in running velocity. All mice not exercised were given access to an immobile treadmill for an equivalent amount of time as the running mice.

Golgi staining

A total of 12 C57BL/6J mouse brains were processed using the Golgi stain. After the final session of running, the C57BL/6 mice were