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Research report

Physical training prevents depressive symptoms and a decrease in brain-derived neurotrophic factor in Parkinson's disease

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ABSTRACT

Depression is a neuropsychiatric disorder that is commonly found in patients with Parkinson's disease (PD). Many studies have suggested that physical exercise can have an antidepressant effect by increasing the levels of brain-derived neurotrophic factor (BDNF), and may also prevent neurodegenerative disease. However, different forms of training may promote different changes in the brain. The aim of this study was to investigate the effects of two types of physical training on depressive-like behavior, and on the levels of proBDNF, BDNF, and its receptor, TrkB, in a mouse model of PD. C57BL/6 mice were subjected to 60 days of exercise: either running on a treadmill or performing a strength exercise. PD was induced by striatal administration of 6-OHDA 24 h after the last physical exercise session. Seven days after 6-OHDA injection, depressive-like behavior and apomorphine-induced rotational behavior were evaluated. The levels of proBDNF, BDNF, and TRKB were measured in the striatum and the hippocampus of mice by immunoblotting assay. The 6-OHDA-treated animals showed a significant increase in immobility time and rotational behavior compared with the control group. In addition, significant decreases in the levels of proBDNF, BDNF, and its receptor, TrkB were observed in the 6-OHDA group. Both types of physical exercise prevented depressive-like behavior and restored the levels of proBDNF, BDNF, and TrkB in the striatum and hippocampus of mice administered 6-OHDA. Our results demonstrate that exercise training was effective for neuroprotection in the striatum and the hippocampus in an experimental model of PD.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder that is characterized by neurodegeneration and loss of dopaminergic neurons. However, other neurotransmitter systems are significantly impaired in this disease due to cell degeneration, including the noradrenergic and serotonergic systems (Chan-Palay and Asan, 1989; Kish, 2003). Together with the severe loss of dopaminergic neurons, the damage to these neurotransmitter systems contributes to the comorbid depression and cognitive deficits that are observed in over 50% of PD patients (Ravina et al., 2007; Martínez-Martín and Damián, 2010; Goldman and Litvan, 2011; Smith et al., 2012).

The pharmacological management of PD is complex, and patients are managed on a case-by-case basis, with the primary aim of controlling symptoms (Worth, 2013). Levodopa is converted to dopamine (DA) in the brain, and is the most effective PD medication for reducing tremor, stiffness, and slowness, and improving muscle control, balance, and walking. However, it is not possible to halt the underlying degeneration, or to treat symptoms that are the result of damage to non-dopaminergic neurons (Minagar et al., 1999; Hirsch, 2012). The pharmacotherapy for depression in PD includes antidepressant drugs, such as serotonin (5-HT) reuptake

Abbreviations: 5-HT, serotonin; 6-OHDA, 6-hydroxydopamine; BDNF, brain-derived neurotrophic factor; DA, dopamine; EDTA, ethylenediaminetetraacetic acid; FST, forced swimming test; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; OFT, open-field test; p75NTR, pan-neurotrophin receptor p75; PD, Parkinson's disease; SNpc, substantia nigra pars compacta; TkrB, tropomyosin related kinase B; PMSF, phenylmethylsulfonyl fluoride; RT, rotational test; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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inhibitors or tricyclic antidepressants; nevertheless, depression increases in both frequency and severity in the later stages of the disease (Coelho and Ferreira, 2012; Starkstein et al., 2012).

For all the reasons mentioned above, novel therapeutic strategies for PD are urgently required for neuroprotection, and to treat symptoms resistant to current treatments. Several studies have indicated that physical exercise can improve quality of life, and prevent or delay the onset of dementia, cognitive decline, or depression (Chen and Weber, 2004; Bower et al., 2010; Miller et al., 2012; Tan, 2012). Furthermore, physical exercise has been related to general health improvements and protection against age-related neurodegenerative disorders, such as Alzheimer's disease and PD (Arcoverde et al., 2008; Goodwin et al., 2008; Lautenschlager et al., 2008; Dibble et al., 2009; Weintraub and Morgan, 2011; Tuon et al., 2012).

Experimental models have been conceived as an important tool for investigating the pathophysiology and developing novel therapeutic strategies for neurodegenerative disorders. In this study, we utilized the injection of 6-hydroxydopamine (6-OHDA) into the terminal region of the striatum in mice, as a classic and widely utilized animal model of PD. In addition, physical training in rodents has been widely used as a therapeutic strategy for neuroprotection in several studies (Marais et al., 2009; Aguiar et al., 2011; Ke et al., 2011; Tuon et al., 2012; Liu et al., 2013), although it must be noted that exercise-induced alterations in the brain are highly specific and depend upon a number of factors, including the type, frequency, intensity, and duration of exercise. Therefore, the mechanisms by which exercise exerts its protective effects still require further investigation. The aim of this study was to investigate the effects of two types of physical training on depressive-like behavior, and on the levels of brain-derived neurotrophic factor (BDNF), proBDNF, and the BDNF receptor, TrkB, in the brains of mice in which PD was induced by 6-OHDA.

2. Experimental procedures

2.1. Animals

We used adult male C57BL mice (25–30 g) obtained from our own breeding colony. The animals were housed five to a cage, on a 12 h light/dark cycle (lights on at 07:00), with free access to food (Nuvilab CR1, Nuvital Nutrientes S/A, Brazil) and water. All experimental procedures were carried out in accordance with the Brazilian Guidelines for the Care and Use of Animals for Scientific and Didactic purposes (DOU 27/5/13, MCTI, p.7), and the local ethics committee approved the study. Six experimental groups were used (each $n = 12$): untrained + operated (Sham), strength training + operated (TSG), treadmill training + operated (TTR), untrained + 6-OHDA (U + 6-OHDA), strength training + 6-OHDA (TSG + 6-OHDA), and treadmill training + 6-OHDA (TTR + 6-OHDA).

2.2. Exercise protocols

2.2.1. Treadmill training

All the animals were habituated on a nine-channel, motor-driven treadmill at a speed of 10 m min^{-1} for 10 min/day for one week to reduce the stress of a new environment. The mice did not receive any stimulus to run. The exercise groups performed an incremental running program to obtain progressive levels of intensity (13–17 m min^{-1}, no incline) three or four days/week for eight weeks, for a total period of 60 days. Each session was of 50 min duration, with a 48 h interval between sessions.

2.2.2. Strength training

This exercise was performed according to Hornberger and Farrar (2004), and entailed climbing a 1 m ladder with a 2 cm grid inclined at 85°. Mice were familiarized with the exercise for three days. Three days after familiarization, the resistance training was begun using cylinders containing weights that were attached to the base of the tail of the mouse with foam tape. Briefly, the cylinders were fastened to the tail by wrapping the upper portion of the tail (2–3 cm from the proximal end) with Velcro on top of the foam tape. Then, the initial weights (50% of body weight) were inserted into the cylinders. The mouse was then positioned at the base of the climbing apparatus and motivated to climb the ladder using a grooming action to the tail. The weight attached to the tail was increased gradually from 50% to 100% throughout the 8 weeks of training: 1st and 2nd wk, 50%; 3rd and 4th wk, 60%; 5th and 6th wk, 80%; 7th and 8th wk, 100%. Three sets of five repetitions, with a 1 min rest between repetitions and 2 min between the sets, were performed for three or four days/week. Each session was of 40–50 min duration, with a 48 h interval between sessions. When the mice reached the top of the ladder, they were allowed to recover in a resting area. This procedure was repeated until either the mice finished all three sets of training, or they failed to climb the entire length of the ladder. The mice were manually stimulated to provide motivation to climb when necessary.

2.3. 6-OHDA injection

Twenty-four hours after the final physical training session, under Equithesin (3 mL/kg i.p.) anesthesia, all animals were placed on a stereotaxic frame. A total of 8 μg of 6-OHDA was administered to each animal (4 μL of a 2 μg/μL solution prepared in 0.2% ascorbic acid and 0.9% saline) from unilateral injections in the terminal region of the striatum, replicating the procedure of Branchi et al. (2010), at bregma coordinates: AP = +1 cm; ML = ±1.7 cm; DV = −2.9 mm, according to the stereotaxic coordinates of Paxinos and Franklin (2000). 6-OHDA was injected via a Hamilton syringe attached to an infusion pump (BI Insight 2000) at a rate of 0.5 μL/min for 8 min. After injection, the needle was left in place for 3 min before slowly retracting it to prevent reflux. Control (Sham) animals received the same volume of vehicle using the identical procedure.

2.4. Behavioral tests: seven days after 6-ohda injection

2.4.1. Open-field test (OFT)

The ambulatory behavior of mice was assessed in the OFT (Kaster et al., 2005; Salehi-Sadaghiani et al., 2012). The apparatus consisted of a wooden box measuring 40 cm × 60 cm × 50 cm. The floor of the arena was divided into 12 equal squares. The animal was gently placed at the center of the field, and the number of squares crossed with all paws (crossing) was counted in a 6 min session. After each test, the apparatus was cleaned with 10% ethanol solution to disguise the presence of other animals. The light inside the apparatus was maintained at a minimum to avoid anxiety-related behavior.

2.4.2. Forced swimming test (FST)

Immediately after the open-field test, the mice were individually placed in an open cylindrical container containing 19 cm of water at 25 ± 1 °C, as previously described (diameter 10 cm, height 25 cm). Mice were allowed to swim for 6 min. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The duration of immobility