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The effects of long-term dopaminergic treatment on locomotor behavior in rats

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ABSTRACT

Long-term treatments with dopaminergic agents are associated with adverse effects, including augmentation. Augmentation consists of an exacerbation of restless legs syndrome (a sleep-related movement disorder) symptoms during treatment compared to those experienced during the period before therapy was initiated. The objective of this study was to examine locomotor activity in rats after long-term dopaminergic treatment and its relationship with expression of the D2 receptor, in addition to demonstrating possible evidence of augmentation. The rats were divided into control (CTRL) and drug (Pramipexole—PPX) groups that received daily saline vehicle and PPX treatments, respectively, for 71 days. The locomotor behavior of the animals was evaluated weekly in the Open Field test for 71 days. The expression of the dopamine D2 receptor was evaluated by Western Blot analysis. The animals that received the PPX demonstrated a significant reduction in locomotor activity from day 1 to day 57 and a significant increase in immobility time from day 1 to day 64 relative to baseline values, but these values had returned to baseline levels at 71 days. No changes in the expression of the D2 receptor were demonstrated after treatment with a dopaminergic agonist. This study suggests changes in locomotor activity in rats after long-term PPX treatment that include an immediate reduction of locomotion and an increase in immobilization, and after 64 days, these values returned to baseline levels without evidence of augmentation. In addition, it was not possible to demonstrate a relationship between locomotor activity and the expression of D2 receptors under these conditions.

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[1]In memoriam. You will always be alive inside each one of us.

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1. Introduction

Long-term treatment with dopaminergic agents is associated with adverse effects, including daytime sleepiness and sleep attacks, impulse control disorder, addiction, augmentation and other effects [1].

Augmentation occurs in up to 60% of restless legs syndrome (RLS) patients treated with levodopa [2] and, to a lesser extent, dopaminergic agonists [3]. This effect was observed in patients as an increase in severity of RLS symptoms with increasing medication doses. Among the features of augmentation are early onset of symptoms, a decrease in latency to the onset of symptoms the during RLS inactivity, symptoms extending to other body parts (e.g., upper limbs and trunk), and reduced efficacy of medication [4]. There are five known factors that predispose patients to augmentation: drugs with a low half-life, dosage increases, long-term treatments [4], low serum ferritin levels [5], and an RLS-positive family history [6].

Previously, studies on augmentation were not directly based on animal models [5]. The vast majority of the research describes augmentation as a result of the chronic treatment of RLS. Animal models are important tools used to verify hypotheses and decipher the details of pathophysiological mechanisms, including the connections between genes, biology and disease. In this context, considering the effectiveness of PPX on the treatment of RLS and taking into account the results of Chernoloz et al. [7] that demonstrate the facilitatory effect of chronic PPX administration on DA neurotransmission, the assessment of the effect of long-term PPX treatment on locomotor activity in rats has been deemed relevant.

Thus, the objective of this research was to examine locomotor activity in rats after long-term dopaminergic treatment and its relationship with the expression of the D2 receptor and to demonstrate possible evidence of augmentation.

2. Materials and methods

2.1. *Animals*

A total of 17 three-month-old male Wistar rats (initial and final weight: 270 g and 380 g, respectively) at the Center for Development of Experimental Models for Medicine and Biology (CEDEME-UNIFESP, São Paulo, Brazil) were used in the experiment. The rats were housed in standard polypropylene cages, maintained in a temperature-controlled room (23 ± 1 °C) with a 12:12-h light–dark cycle (lights on at 7 a.m.) and had access to food and water ad libitum. The experimental protocol was approved by the Ethics Committee of UNIFESP (CEP: 0881/11). The animals were divided into control group ($n=8$) and drug groups ($n=9$).

2.2. *Experimental design*

Initially, the animals underwent adaptation to the Open Field (10 min). After the adaptation period and baseline test, treatment was initiated, and the animals received a daily dose of either the drug or saline solution at the same time each day for 71 days. Tests were conducted in the Open Field on days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, and 71 of treatment. The treatment occurred between 7 a.m and 8 a.m on days when the animals did not participate in the Open Field test and between 7 a.m and 10:30 a.m on days when they were evaluated in the Open Field. After the last Open Field test on day 71 of treatment, the animals were euthanized, and the striatum was extracted.

2.3. *Pharmacological treatment*

The treatment consisted of a saline vehicle at 0.9% (0.1 mL/100 g) for the control group and pramipexole (0.1 mg/kg, dissolved in the saline solution at 0.9%) for the drug group and was always administered at the same time of day for 71 days [8]. On days when rats were evaluated in the Open Field, the dose was injected into the peritoneal cavity 10 min before the beginning of the test.

2.4. *Open Field*

The exposure to the Open Field [9] was performed between 9 a.m. and 11 a.m., and each animal was individually placed in the center of the apparatus and observed for 10 min. However, only the final 5 min was evaluated. The Open Field consisted of a circular wooden ring measuring 81 cm in diameter and enclosed by 41 cm-high white walls. The ceiling was open, and the ground was divided into 19 quadrants. In line with the circadian pattern of human RLS symptoms, which appear or worsen during the night, all tests were performed between 9 a.m. and 11 a.m. because rats are nocturnal animals [10]. During the experiment, the rats were evaluated for peripheral ambulation (number of quadrants that the animal stepped on with four paws near the walls of the apparatus), central ambulation (number of quadrants that the animal stepped on with four paws that were not close to the walls of the apparatus), total ambulation (the total number of quadrants that the animal stepped on with four paws) rearing (the number of times that the animal supported itself with both hind legs), total duration of grooming (the total amount of time that the animal put its mouth or paws on its body or head) and immobility time (the total amount of time that the animal remained perfectly still, moving only the vibrissae) [11].

2.5. *Western Blot*

After decapitation, the striatum (caudate and putamen) was rapidly removed, and the fragments were frozen. During the dissection, the equipment was kept at a low temperature, and the biological material was stored at −80 °C until used. D2 dopaminergic receptor and the dopamine transporter (DAT) expressions were evaluated by Western Blot analysis. The tissue was homogenized in lysis buffer (1% Triton X-100, 0.5% sodium deoxycholate, 100 mM Tris–HCl pH 8.0, 150 mM NaCl, 10 mM EDTA, 0.1% SDS, 10% glycerol, and Protease Inhibitor Cocktail (Sigma), incubated on ice for 10 min, centrifuged at 7000*g* for 5 min at 4 °C, and the supernatant was collected. Protein concentration was assayed using the bicinchoninic