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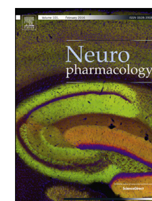

# Contrasting gene expression patterns induced by levodopa and pramipexole treatments in the rat model of Parkinson's disease

Irene R. Taravini [a, *, 1], Celia Larramendy [a, 1], Gimena Gomez [a], Mariano D. Saborido [a], Floor Spaans [b], Cristóbal Fresno [c], Germán A. González [c], Elmer Fernández [c], Mario G. Murer [d], Oscar S. Gershanik [a]

[a] Laboratorio de Parkinson Experimental, Instituto de Investigaciones Farmacológicas (ININFA-CONICET-UBA), Ciudad Autónoma de Buenos Aires, Argentina
[b] Division of Medical Biology, Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[c] Facultad de Ingeniería, Universidad Católica de Córdoba, CONICET, Córdoba, Argentina
[d] Laboratorio de Fisiología de Circuitos Neuronales, Instituto de Fisiología y Biofísica (IFIBIO Houssay), CONICET. Facultad de Medicina, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

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Whether the treatment of Parkinson's disease has to be initiated with levodopa or a D2 agonist like pramipexole remains debatable. Levodopa is more potent against symptoms than D2 agonists, but D2 agonists are less prone to induce motor complications and may have neuroprotective effects. Although regulation of plastic changes in striatal circuits may be the key to their different therapeutic potential, the gene expression patterns induced by *de novo* treatments with levodopa or D2 agonists are currently unknown. By studying the whole striatal transcriptome in a rodent model of early stage Parkinson's disease, we have identified the gene expression patterns underlying therapeutically comparable chronic treatments with levodopa or pramipexole. Despite the overall relatively small size of mRNA expression changes at the level of individual transcripts, our data show a robust and complete segregation of the transcript expression patterns induced by both treatments. Moreover, transcripts related to oxidative metabolism and mitochondrial function were enriched in levodopa-treated compared to vehicle-treated and pramipexole-treated animals, whereas transcripts related to olfactory transduction pathways were enriched in both treatment groups compared to vehicle-treated animals. Thus, our data reveal the plasticity of genetic striatal networks possibly contributing to the therapeutic effects of the most common initial treatments for Parkinson's disease, suggesting a role for oxidative stress in the long term complications induced by levodopa and identifying previously overlooked signaling cascades as potentially new therapeutic targets.

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*Abbreviations:* 6-OHDA, 6-hydroxydopamine; DA, dopamine; Gabarap, Gamma-aminobutyric acid receptor-associated protein; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; GPx, glutathione peroxidase; HPRT, Hypoxanthine-guanine phosphoribosyltransferase; KEGG, Kyoto Encyclopedia of Genes and Genomes; LES-LEVO, group of lesioned rats treated with levodopa; LES-PRAMI, group of lesioned rats treated with pramipexole; LES-VEH, group of lesioned rats treated with vehicle; Mybl1, myeloblastosis oncogene-like 1; Ndufa12, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 12; NORM-VEH, group of normal rats treated with vehicle; Nr4a2, Nurr1, nuclear receptor subfamily 4, group A, member 2; Olr1375, olfactory receptor 1375; PBS, phosphate-buffered saline; PD, Parkinson's disease; Ppp1r2, Protein phosphatase inhibitor 2; Psmd14, proteasome (prosome, macropain) 26S subunit, non-ATPase, 14; qRT-PCR, quantitative real-time RT-PCR; SHAM, rats injected with vehicle instead of 6-OHDA; SNpc, substantia nigra pars compacta; Sod1, superoxide dismutase 1; TH, tyrosine hydroxylase; TH-ir, TH-immunoreactive.

* Corresponding author. Laboratorio de Parkinson Experimental, Instituto de Investigaciones Farmacológicas, Facultad de Farmacia y Bioquímica. Junín 956 5° piso, (C1113AAD), Ciudad Autónoma de Buenos Aires, Argentina. Tel.: +54 11 4961 5949; fax: +54 11 4963 8593.

*E-mail addresses:* taravini@ffyb.uba.ar (I.R. Taravini), celialarramendy@gmail.com (C. Larramendy), gimenagomez@gmail.com (G. Gomez), marianosaborido@gmail.com (M.D. Saborido), f.spaans@umcg.nl (F. Spaans), cfresno@bdmg.com.ar (C. Fresno), ggonzalez@bdmg.com.ar (G.A. González), efernandez@bdmg.com.ar (E. Fernández), gmurer@gmail.com (M.G. Murer), gersha@gmail.com (O.S. Gershanik).

[1] Co-first authors, contributed equally.

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# 1. Introduction

Levodopa and pramipexole are two of the most frequently used agents for the symptomatic treatment of Parkinson's disease (PD). These two drugs differ significantly in terms of their pharmacokinetic and pharmacodynamic profiles. Levodopa is a pro-drug that needs to be enzymatically converted into dopamine (DA) within the brain to be able to stimulate all DA receptors (Cotzias et al., 1969). Pramipexole acts directly on DA receptors of the D2 family with special affinity for D3 receptors (Bennett and Piercey, 1999). Their half-lives are quite different; pramipexole has a half-life of more than six hours, while levodopa has a very short half-life of less than two hours. These differences account in part for their clinical effects. Levodopa is the most potent in terms of symptomatic improvement, but its short half-life and its effects on both DA receptors subfamilies (D1 and D2) are believed to be responsible for the development of troublesome motor complications after long-term treatment, namely motor fluctuations and dyskinesias (Cenci and Konradi, 2010; Jenner, 2008; Murer and Moratalla, 2011). Pramipexole, on the other hand, while showing less potency in the improvement of motor symptomatology, is less prone to the development of motor complications and might delay and reduce them when used initially in monotherapy in "*de novo*" patients (Holloway et al., 2004; Parkinson Study Group, 2002, 2000). In addition, for a long time, a controversy on the existence of differential effects on the survival of remaining DA neurons, between these two drugs, has divided the opinions of experts in the field. Levodopa was thought to carry the risk of promoting cell death, through the increase of oxidative by-products within remaining DA neurons, while pramipexole was proposed to have neuroprotective properties. A cumulative body of evidence has been produced that helped to dispel the concept of levodopa toxicity, (Murer et al., 1999, 1998; Olanow et al., 2004), while on the other hand has casted doubts on the putative neuroprotective properties of pramipexole (Schapira et al., 2013). Furthermore, significant behavioral, biochemical and molecular differences induced by early *versus* delayed administration of levodopa or pramipexole in hemiparkinsonian rats have been reported (Marin et al., 2014). These evidences notwithstanding, there is still much to learn in regards to the ultimate mechanism of action of these two drugs and which additional factors account for their differences in terms of clinical effects.

In recent years, much has been learned about the cascade of molecular events that are set in motion downstream of the DA receptors both in the context of denervation of the nigrostriatal system by the pathological process, and by the non-physiologic stimulation of denervated DA receptors by either DA (through exogenous replacement by levodopa) and by DA receptor agonists (Ferrario et al., 2004; Grünblatt et al., 2011; Konradi et al., 2004; Meurers et al., 2009). These pervasive changes involving stimulation of transcription factors, differential expression of genes and their corresponding proteins are believed to be responsible for some of the enduring changes that underlie the development of motor complications and perhaps for their putative neuroprotective or disease modifying effects. Here we looked at the whole striatal transcriptome to characterize the gene expression patterns underlying the therapeutic effects of chronic treatments with levodopa or pramipexole in rats with nigrostriatal lesions.

# 2. Materials and methods

## 2.1. Animals

Male Wistar rats weighing 200–220 g at the beginning of the experiments were purchased from Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (Buenos Aires, Argentina). Rats were caged in groups of three or four, with free access to food and tap water in a temperature-controlled room (20 ± 2 °C) with a 12 h light/dark cycle (light period from 8 a.m. to 8 p.m.). All surgical procedures were performed in accordance with European Union Directive 2010/63/EU guidelines for the use and care of laboratory animals, as well as Argentine regulations (RS617/2002, Servicio Nacional de Sanidad y Calidad Agroalimentaria, SENASA, Argentina). All studies complied with the ARRIVE guidelines. All efforts were made to minimize animal suffering and to reduce the number of animals used.

## 2.2. Drugs

Commercially available Levodopa/carbidopa 250/25 mg (Lebocar, Pfizer SRL, Argentina) and Pramipexole 1 mg (Sifrol, Boehringer-Ingelheim, Germany) were dissolved in tap water as vehicle, filtered, and made available to rats in light protected bottles. This was the animals' only source of fluid and was prepared three times a week. The stability of levodopa in tap water was determined in a previous work (Ferrario et al., 2004). The concentration of the pramipexole solution remained unaltered after four days in tap water as determined by high-performance liquid chromatography (not shown). To keep the doses constant during the three weeks of treatment, drug concentration was readjusted to the mean weight of the animals and the volume of liquid they drank (Datla et al., 2001; Ferrario et al., 2004).

## 2.3. Intrastriatal 6-hydroxydopamine lesion

In order to produce a protracted extensive degeneration of the nigrostriatal pathway, an intrastriatal 6-OHDA injection was performed following a protocol described by Kirik et al. (1998) with slightly variations. Under deep anesthesia with ketamine/xylazine 60/10 mg/kg, respectively (Ketamina 50, Holliday–Scott, Argentina and Xylazine, Kensol, König, Argentina), rats received three stereotaxic injections of 8 μg of 6-hydroxydopamine hydrobromide (calculated as free base) (6-OHDA, MP Biochemicals, USA) dissolved in 3 μl of 0.02% ascorbic acid in saline in the left striatum with a 30-gauge steel cannula (lesioned group, LES). The dose used was selected on the basis of experience from previous experiments (not shown). A group of control rats received vehicle (0.02% ascorbic acid in saline) instead of 6-OHDA (SHAM group). The injection rate was 0.55 μl/min and the cannula was left in place for additional 3 min before slowly retracting it. Stereotaxic coordinates from bregma (mm) were: (1): 1.0 anterior, 3.0 lateral, 5.0 ventral; (2): 0.1 posterior, 3.7 lateral, 5.0 ventral; (3): 1.2 posterior, 4.5 lateral, 5.0 ventral. Rats were placed on a heating pad to minimize hypothermia until they recovered from anesthesia.

## 2.4. Behavioral evaluation

Akinesia of the contralateral forepaw was assessed in limb-use asymmetry tests, the cylinder test (Schallert et al., 2000) and the stepping test (Olsson et al., 1995). In the cylinder test a rat is placed in a transparent acrylic cylinder (20 cm diameter, 30 cm height) and the observer counts the number of wall contacts performed with the left, right, or both forelimbs simultaneously, during 5 min of spontaneous vertical exploration. An asymmetry score was calculated as percentage of the number of contralateral forelimb wall contacts plus 1/2 the number of both forelimbs wall contacts, divided by the total number of wall contacts (ipsilateral plus contralateral plus both forelimb contacts) (Larramendy et al., 2008; Woodlee et al., 2005). The cylinder test was performed three days before and two weeks after surgery in order to select the successfully lesioned animals. On the third week of the pharmacological treatments this test was carried out from 10 p.m. to 6 a.m., period of maximum activity of animals and maximum consumption of the drugs solution. The stepping test was conducted twice a day for three consecutive days (the animals were handled during 2 days to become familiar with the manipulation). The rat was held in one hand fixing the hindlimbs whereas one of the forelimbs was slightly fixed with the other hand. In this position and with the other forepaw touching the surface of a table of 90 cm, the rat was moved in 5 s, first to the forehand and then to the backhand direction. The number of adjusting steps was counted in both directions for each forelimb and a mean value was obtained by averaging the number of steps observed across the six sessions (theoretical maximum score per session: 16). Abnormal involuntary movements or "dyskinesias" were measured as previously described using the following scale: 0: absent; 1: occasional; 2: frequent; 3: continuous interrupted by sensory distraction; 4: continuous not interrupted by sensory distraction (Cenci and Lundblad, 2007; Delfino et al., 2004; Larramendy et al., 2008; Lundblad et al., 2004).

## 2.5. Tissue preparation for immunohistochemistry

Rats were anesthetized with ketamine/xylazine (60/10 mg/kg, i.p.) and perfused transcardially with 100 ml of saline followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Brains were post-fixed for 2 h in the same fixative solution, cryoprotected in 30% sucrose in 0.1 M PBS for 48 h and stored at 4 °C until sectioning. Serial coronal, 40-μm-thick tissue sections of striatum and *substantia nigra pars compacta* (SNpc) were cut in a freezing microtome. The slices were stored in PBS containing 0.1% sodium azide at 4 °C. Animals under three weeks of pharmacological treatment were perfused after a drug washout period of 24 h.

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