



Rotenone impairs hippocampal neuronal activity in a rat model of Parkinson's disease



L.V. Darbinyan^a, L.E. Hambardzumyan^a, K.V. Simonyan^{b,*}, V.A. Chavushyan^b,
L.P. Manukyan^a, V.H. Sarkisian^a

^a Sensorimotor Integration Lab, Orbeli Institute of Physiology NAS RA, Yerevan, 0028, Armenia

^b Neuroendocrine Relationships Lab, Orbeli Institute of Physiology NAS RA, Yerevan, 0028, Armenia

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ABSTRACT

Parkinson's disease (PD) is the most common neurodegenerative disease of unknown etiology and characterized by motor symptoms of tremor, rigidity, bradykinesia, and postural instability. Interactions between the dopaminergic systems and the hippocampus in synaptic plasticity and behavior are found. The rotenone-induced animal model is commonly used in research studies involved in PD. Administration of rotenone causes alterations of electrical neuronal activity. Rotenone (2.5 mg/kg/day) was administered intraperitoneally for 21 days to adult rats, and rotenone effects on rearing activity and electrophysiology were examined. Dose-dependent reduction of evoked neural activity and a reduction in firing strength were found in the hippocampus. Behaviorally, Rotenone rats showed a more consistent decrease in rearing across the 3 weeks, compared with animals in the control group. Thus, rotenone causes changes in hippocampal electrical activity and behavioral changes.

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1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative disease of unknown etiology and characterized by motor symptoms of tremor, rigidity, bradykinesia, and postural instability. Parkinson's disease is characterized by an abnormal basal ganglia activity. Non-motor comorbidities, such as cognitive impairments (the comorbidity of anxiety and depression in Parkinson's disease) are likely the result of an intricate interplay of multi-system degenerations and neurotransmitter deficiencies extending beyond the loss of dopaminergic nigral neurons. Cholinergic denervation may occur early in PD [1]. Recently, increased attention has been directed towards the hippocampus in the development of non-motor symptoms [2]. The etiology of PD has not been completely understood yet. The causes of the disorder are likely to be multiple and to involve not only single factors alone, but also several factors acting together [3]. Epidemiological studies suggest that exposure to some toxic agents such as pesticides may increase PD risk and cause some symptoms of PD [4]. The PD patients are characterized by systemic mitochondrial dysfunction, marked by inhibition of complex I of the mitochondrial electron

transport chain (METC) and impairment of all ATP-dependent cellular processes. Selective inhibitors of METC are known to produce neuronal injury by secondary excitotoxic mechanisms. Rotenone, prototypic mitochondrial poison, is a selective inhibitor of complex I of METC [5]. Rotenone-induced animal model is commonly used in research studies involved in PD [6,7]. Patients with Parkinson's disease may have hippocampal atrophy and progressive hippocampal volume loss compared with controls [8]. Rotenone rat model is one of well-known models of PD [9]. The insecticide rotenone is highly lipophilic, so it readily crosses the blood–brain barrier and diffuses into neurons where, in a manner similar to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), it accumulates within mitochondria and inhibits complex I. The ensuing reductions in ATP are not, however, considered a cause of the toxicity; rather the production of ROS (reactive oxygen species) subsequent to glutathione depletion is thought to induce oxidative stress [4]. Oxidative damage, in the form of protein carbonyl formation, has certainly been found in the midbrain, olfactory bulb, striatum and cortex of rats treated with rotenone [4]. The extensive microglial activation seen in both the SNc (substantia nigra compacta) and striatum following rotenone infusion [10] is consistent with the inflammatory features found in idiopathic PD [11,12] lending support to the construct validity of this model. Rotenone also inhibits proteasomal activity [13]. Rotenone model may show promise for selecting agents with potential neuroprotective effi-

* Corresponding author.

E-mail address: karensimonyan86@yandex.ru (K.V. Simonyan).

ciency [14,15]. The goal of this study was to characterize a rotenone model of PD and the hippocampal degeneration. Here we present the experimental procedures of the model and characterization of its *in-vivo* electrophysiological and behavioral features.

2. Materials and methods

2.1. Animals

Adult male albino rats weighing 200 ± 30 g were purchased from the experimental center of Orbeli Institute of Physiology NAS RA. The animals were maintained at $25 \pm 2^\circ\text{C}$, 12 h light – dark cycle and lights on 07:00–19:00 h. Food and water *ad libitum* was provided to the animals. All experiments were carried out in separate and isolated laboratories, which have the environmental conditions same as the colony room. The experiments were performed at the same time-period of the day (09:00–12:00 h) and during the light period of the light–dark cycle. All of the experimental protocols were approved by the Committee of Ethics of the Yerevan State Medical University (YSMU) (Yerevan, Armenia), followed the “Principles of laboratory animal care” and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Reagents

Rotenone was purchased from Sigma (St. Louis, MO, USA) and dissolved in sterile Sunflower oil. Rotenone dissolved in Sunflower oil at 2.5 mg/ml, was given intraperitoneally (i.p.), daily at 1 ml/kg for 3 weeks. Sunflower oil alone was injected to the control rats (1 ml/kg).

2.3. Study design

Rats were randomly divided into 3 groups, each having 6 animals: vehicle (Sunflower oil)-control group; experimental rats: i.p. injections of the vehicle in a volume of 1 ml/kg with rotenone; (rotenone group); and the norm (untreated) group.

2.4. Cylinder test

This test aims to determine the presence and extent of movement lateralization indicative on unilateral neurological damage. The animals were placed in a clear Plexiglas cylinder (20 in cm diameter and 30 cm in height) in order to evaluate motor asymmetry. A mirror was placed to the side of the cylinder at an angle to enable the recording of forelimb movements even when the animal was turned away from the camera. Scoring was done by an experimenter blinded to the condition of the animal using a video cassette recorder with slow-motion and clear stop-frame capabilities. A video camera above the field was connected to a video recorder and a monitor, recording the movement of the rat. During rearing, behavior, the forelimbs will contact the wall of the cylinder. Rats were tested only once to prevent habituation to the apparatus. To be classified as a rear, the animal had to raise forelimbs above shoulder level and make contact with the cylinder wall with either one or both forelimbs. Removal of both forelimbs from the cylinder wall and contact with the table surface was required before another rear was scored. Forelimb contacts while rearing are scored with a total of 5 min for each animal. Data of all the results were presented as mean $\pm$ SEM. Significant differences between groups were calculated using Student's *t*-test and $p < 0.05$ was considered statistically significant.

2.5. In vivo electrophysiology and data analysis

In acute experiment the animals were anesthetized (Urethan 1.2 g/kg), immobilized with 1% ditiline (25 mg/kg i/p), fixed in a stereotaxic head frame and were transferred to artificial respiration. The sample of isolated rat brain was obtained by transection of spinal cord (T2–T3). The stimulating electrode was repeatedly inserted into the ipsilateral entorhinal cortex (EC) according to stereotaxic coordinates [16] (AP – 9, $L \pm 3.5$, DV +4.0 mm) and a glass recording electrode (1–2 μm tip diameter) filled with 2 M NaCl was repeatedly inserted into the hippocampal fields at coordinates (AP–3.2–3.5; $L \pm 1.5$ –3.5; DV +2.8–4.0 mm) for recording spike activity flow of single neurons. High frequency stimulation (HFS) (100 Hz during 1 s) was performed by means of rectangle pulses of 0.05 ms duration and 0.08–0.16 mA amplitude. Electrophysiological recordings and mathematical analysis of spike activity were accomplished using an automated analysis program (V.S. Kamenetski) providing selection of spikes by amplitude discrimination, which pinpoints spikes and excludes artifacts during HFS, allowing not only posttetanic, but also tetanic activity evaluation [17]. The timing, frequency and cumulative histograms, as well as a diagram of mean frequency for single neurons and populations of neurons with uniform responses were constructed on the basis of analysis of peristimulus spiking. For statistical evaluation we used *t*-criteria of Student's *t* – test, the reliability of differences of interspike intervals before, after and during HFS. To increase reliability of statistical evaluations, we also used the non-parametric method of verification by application of Wilcoxon two-sample test taking into account the asymptotic normality of this criterion and allowing comparison of the calculated values with the table values of the standard normal distribution (at the significance levels 0.05, 0.01, and 0.001). Tetanic potentiation (TP) or tetanic depression (TD) and following posttetanic potentiation (PTP) and posttetanic depression (PTD) were recorded to HFS of ipsilateral EC.

3. Results

An electrophysiological analysis in control group showed (Fig. 1) that tetanic potentiation during HFS (100 Hz) in the hippocampus with TP PTP expressed 4.12 times (53.96: 13.10 spike/sec), TP PTD responses – 2.71 times (28.39: 10.47 spike/sec). Share of hippocampal neurons with TP is predominant (21.97%) (Table 1). Tetanic depression during HFS (100 Hz) in neurons with TD PTD expressed 3.38 times (24.03: 7.11 spike/sec), TD PTP responses – 1.58 times (20.08: 12.75 spike/sec) (Fig. 1), TD 5.2 times (7.78: 1.5 spike/sec), nonreactive neurons (5.3%).

In the rotenone group, the tetanic potentiation expressed 1.7 times (15.76: 9.36 spike/sec), TP PTD responses 1.86 times (18.18: 9.77 spike/sec) (Fig. 2).

In the norm group (Fig. 3) tetanic potentiation during HFS in hippocampal neurons with TP PTP expressed 3.94 times (32: 8.11 spike/sec), tetanic depression during HFS in neurons with TD PTD responses – 8.82 times (6.53: 0.74 spike/sec), TD PTP responses – 8.38 times (5.78: 0.69) (Fig. 3). In the norm group the share of neurons exhibiting TD PTD responses were dominant (42.04%, Table 3). Inhibitory (tetanic depression) TD PTD and TD PTP responses expressed respectively by 1.69-fold (10.70: 6.35 spike/sec) and 2.06-fold (11.71: 5.68 spike/sec), PTP responses 1.39 times, TP (6.34%), TD 1.72 times (Fig. 2), nonreactive neurons (15.34%). The share of hippocampal neurons with TD PTP and TP PTP responses is predominant (20.1%, Table 2). In general, during HFS (M HFS) in control and rotenone groups the nonreactive neuron are 5.3% and 15.34%, Table 2). Nonreactive neurons in group control group show the lowest share.

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