Neuroscience Letters 513 (2012) 187–192

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Functional neuroimaging of the 6-OHDA lesion rat model of Parkinson's disease

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ARTICLE INFO

Article history:
Received 29 August 2011
Received in revised form 26 January 2012
Accepted 12 February 2012

Keywords:
[18F]-fluoro-2-deoxy-D-glucose
6-Hydroxydopamine
Parkinson's disease
Positron emission tomography

ABSTRACT

We characterized the unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rat, a well-known acute model of Parkinson's disease (PD), with [^{18}F]-fluoro-2-deoxy-D-glucose (FDG) small-animal positron emission tomography (PET), which we compared with a drug-induced rotation behavioral test. In the 6-OHDA model, significant glucose hypometabolism was present in the primary motor cortex, substantia nigra, and pedunculopontine tegmental nucleus on the ipsilateral side. In contrast, neuronal activations were observed in the primary somatosensory cortex and ventral caudate–putamen area after lesioning. Correlation analysis revealed a significant relationship between the behavioral results and the degree of glucose metabolism impairment in the primary motor cortex, substantia nigra, and pedunculopontine tegmental nucleus. In addition, the pedunculopontine tegmental nucleus correlated significantly with the primary somatosensory cortex, the ventral caudate–putamen, the substantia nigra, and the primary motor cortex. Furthermore, the primary motor cortex also showed significant correlations with the substantia nigra. In conclusion, *In vivo* cerebral mapping of the 6-OHDA-lesioned rats using [^{18}F]-FDG PET showed correspondence at the functional levels to the cortico–subcortical network impairment observed in PD patients.

1. Introduction

Parkinson's disease (PD) is a chronic, progressive, neurodegenerative disorder characterized by the massive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc). This nigral neuronal loss leads to a striatal dopamine deficiency, which is considered to underlie the symptoms of the disease.

Several phenotypical features of human PD can be simulated in rodents with the intracerebral injection of neurotoxins [3]. A widely used experimental model of acute PD relies on the administration of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB), which selectively destroys catecholaminergic neurons through mitochondrial damage [7]. The neurochemical and histopathological changes induced by the unilateral injection of 6-OHDA into the medial forebrain bundle (MFB) have been extensively characterized using invasive histological and electrophysiological analyses [28]. On the behavioral-motor levels, characteristic gait disturbances are easily assessable using tests that examine side bias, such as apomorphine-induced rotation tests [28].

The recent development of positron emission tomography (PET) scanners designed for laboratory animals allows the further detailed study of rodent models of human neurodegenerative diseases by providing *in vivo* insights into the biochemical and molecular processes involved and permitting noninvasive follow-up with neuromodulatory approaches.

2-[^{18}F]-fluoro-2-deoxy-D-glucose ([^{18}F]-FDG) is used as a marker of cerebral glucose consumption based on neuronal entrapment and the accumulation of [^{18}F]-FDG-6-PO_4, which indicate neuronal activity [32]. In PD patients, specific cortico–subcortical metabolic changes have been described based on direct regional analysis [17] or network analysis approaches [20]. [^{18}F]-FDG imaging also contributes clinically to the differential diagnosis of Parkinsonian disorders [8]. Although a number of *in vivo* imaging studies of dopaminergic neurotransmission in animal models of PD have been published [13,14,30], there are few studies on the glucose metabolic changes *in vivo* in unilateral 6-OHDA-lesioned, freely moving, conscious rats, which would further validate the biochemical extrapolation to the cortico–subcortical changes observed in humans [6].

Therefore, the aim of this study was to characterize the unilateral 6-OHDA lesioned rat model using [^{18}F]-FDG imaging. This

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doi:10.1016/j.neulet.2012.02.034

allowed us to obtain serial brain images in the same subjects for correlation with behavioral measurements (apomorphine-induced rotation test).

2. Materials and methods

2.1. Animal handling

Thirteen healthy adult male Sprague Dawley rats (300–350 g; Koatech, Pyeongtaek, Korea) were used (unilateral 6-OHDA-lesioned rats). All procedures were performed in accordance with the US National Institutes of Health Guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals). The animals were housed under a 12 h light/dark cycle (lights on at 0800) and 50–60% humidity, with access to food and water *ad libitum*. The animals were acclimatized to the laboratory environment for seven days before the experiments were begun.

2.2. Unilateral 6-OHDA lesioning procedure

All rats subjected to 6-OHDA lesioning of the MFB were anesthetized with tiletamine–zolazepam (2.5 mg/kg) and xylazine hydrochloride (0.3 mg/kg). All rats had been treated with desipramine (25 mg/kg, i.p.) to protect their noradrenergic neurons before 6-OHDA lesioning. A burr hole was trephined in the right frontal bone using a micro drill. A Hamilton syringe was used to inject 12 μg of 6-OHDA (Sigma) dissolved in 4 μL of sterile 0.9% NaCl and 0.02% ascorbic acid into the MFB at a flow rate of 0.5 μL/min. The stereotaxic coordinates of the injection site were anteroposterior −4.4 mm, lateral 2.1 mm, and dorsoventral −8 mm, according to the stereotaxic atlas of Paxinos and Watson [26]. After the injection, the needle was withdrawn slowly, over 10 min and the animals allowed to recover from the anesthesia. They were returned to the animal facility for four weeks, by which time the degeneration of the dopaminergic neurons induced with the neurotoxin had stabilized.

2.3. [^{18}F]-FDG microPET imaging

The experimental design is shown in Fig. 1(a). One week before the unilateral 6-OHDA lesion procedure, baseline microPET scans were performed in all rats in the normal state. PET scans were then performed four weeks after 6-OHDA lesioning to assess the status of the PD model. [^{18}F]-FDG (500 μCi/100 g body weight) was administered by tail vein injection without anesthesia. The temperature in the cages was maintained at 30 °C throughout the uptake period (30 min), in accordance with the optimized [^{18}F]-FDG uptake protocol [11], and was followed by PET scanning for 30 min. Metabolic imaging was performed using a Focus 120 MicroPET system (Concorde Microsystems, Knoxville, TN). During the [^{18}F]-FDG scans, the animals were kept under anesthesia induced with isoflurane inhalation (2% in 100% oxygen; IsoFlo; Abbott Laboratories) and placed on a heating pad maintained at 30 °C. The images were reconstructed using the ordered subset expectation maximization algorithm.

2.4. Behavioral test (apomorphine-induced rotation test)

The drug-induced rotational response was measured in a white, hemispheric, plastic rotation bowl (42 cm wide at the top and 22 cm deep) five weeks after the 6-OHDA MFB lesions were induced. The rats were given 0.5 mg/kg R-apomorphine hydrochloride (Sigma) dissolved in 0.1% ascorbate–saline, and placed immediately in the rotation bowl. The numbers of rotations in the ipsilateral and contralateral directions were counted and expressed as net turns per hour (the number of contralateral turns minus the number of ipsilateral turns).

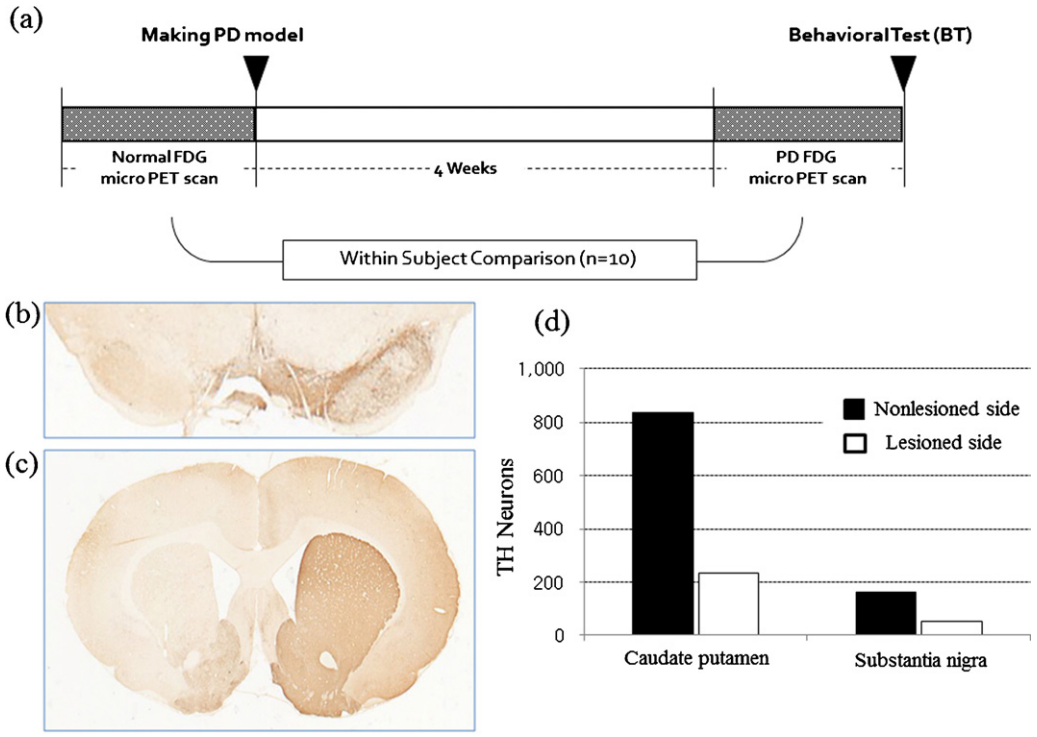

Fig. 1. Experimental protocol and immunohistochemical tyrosine hydroxylase (TH) staining in the substantia nigra and caudate–putamen. (a) Experimental protocol. A unilateral 6-hydroxydopamine (6-OHDA) lesion resulted in profound dopaminergic cell loss in the substantia nigra (a) and the concomitant depletion of TH-positive fibers in the caudate–putamen (b) in the ipsilateral hemisphere. (c) Mean numbers of TH-immunoreactive neurons in each brain region.