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Research report

Sensorimotor assessment of the unilateral 6-hydroxydopamine mouse model of Parkinson's disease

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ABSTRACT

Parkinson's disease (PD), the second most common neurodegenerative disorder, is characterized by marked impairments in motor function caused by the progressive loss of dopaminergic neurons in the substantia nigra *pars compacta* (SNc). Animal models of PD have traditionally been based on toxins, such as 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), that selectively lesion dopaminergic neurons. Motor impairments from 6-OHDA lesions of SNc neurons are well characterized in rats, but much less work has been done in mice. In this study, we compare the effectiveness of a series of drug-free behavioral tests in assessing sensorimotor impairments in the unilateral 6-OHDA mouse model, including six tests used for the first time in this PD mouse model (the automated treadmill "DigiGait" test, the challenging beam test, the adhesive removal test, the pole test, the adjusting steps test, and the test of spontaneous activity) and two tests used previously in 6-OHDA-lesioned mice (the limb-use asymmetry "cylinder" test and the manual gait test). We demonstrate that the limb-use asymmetry, challenging beam, pole, adjusting steps, and spontaneous activity tests are all highly robust assays for detecting sensorimotor impairments in the 6-OHDA mouse model. We also discuss the use of the behavioral tests for specific experimental objectives, such as simple screening for well-lesioned mice in studies of PD cellular pathophysiology or comprehensive behavioral analysis in preclinical therapeutic testing using a battery of sensorimotor tests.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra *pars compacta* (SNc), which leads to severe motor impairments, including bradykinesia, akinesia, muscular rigidity, altered gait, resting tremor, and postural instability [1,2]. PD is primarily a 'sporadic' disease and although a number of environmental and genetic risk factors have been identified, the exact cause in the majority of cases remains unknown [2–4].

A classic animal model of PD, used for over 40 years, is based on intracranial injection of the toxin 6-OHDA in the rat, which leads to a selective loss of nigrostriatal dopaminergic neurons [2,5–7]. The intracranial route of delivery is necessary since 6-OHDA does not cross the blood brain barrier [2,8,9]. To model PD conditions, 6-OHDA can be injected either into the SNc, the medial forebrain bundle (MFB) containing the ascending nigrostriatal fibers, or the striatum. 6-OHDA injections in the MFB or SNc produce a near complete lesion of nigrostriatal neurons that is comparable to neuronal loss in late-stage PD patients. In contrast, 6-OHDA injections in the striatum yield a more progressive and less extensive lesion of nigrostriatal neurons, which may better emulate earlier stages of PD [2,10]. 6-OHDA injections are typically done unilaterally, which allows for an easy comparison of motor impairment between the contralateral, impaired side *vs.* the ipsilateral, unimpaired side of the body. The unilateral 6-OHDA rat model has been widely used in studies of PD pathophysiology [11–16] and for preclinical testing of therapeutic approaches such as deep brain stimulation [17–19] and gene therapy for late-stage PD [20–25].

Behavioral assessments of motor impairments in the unilateral 6-OHDA rat model were initially done by amphetamine- or apomorphine-induced rotation tests [26]. However, since repeated administration of psychostimulants causes changes in synaptic function and dendritic morphology [27,28], the use of drug-induced rotation tests has been replaced in many studies by drug-free sensorimotor behavioral tests. For example, unilateral 6-OHDA-lesioned rats show robust limb-use asymmetry in the cylinder test, akinesia in the stepping test, altered stride length in gait analysis,

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and impairments in the adhesive removal test [29–33]. Recently, many laboratories have begun to use mice in PD research because of the availability of mouse models carrying genetic mutations linked to familial forms of PD [34–36] and transgenic mice expressing fluorescent proteins targeted to different cell types in the basal ganglia [37]. Therefore, we wanted to establish a comprehensive set of behavioral tests to measure sensorimotor impairments in 6-OHDA mice that can be applied to future PD studies with 6-OHDA-lesioned mice.

2. Materials and methods

2.1. Animals

All animals used in this study were wildtype male C57BL/6 mice (Charles River). All procedures were conducted in accordance to protocols approved by the Northwestern University Animal Care and Use Committee (ACUC), and were in compliance with the US National Institutes of Health (NIH) Guide to the Care and Use of Laboratory Animals. The mice were provided with food and water *ad libitum* and kept in 12-h light/dark cycles.

2.2. 6-OHDA and saline MFB injections

6-Hydroxydopamine and saline injections were performed in male C57BL/6 mice at 3–5 weeks of age. The surgical procedure for 6-OHDA and saline injections was modified based on previously established protocols for 6-OHDA injections in rats [12] and for general stereotaxic injections in mice [38]. Animals were anesthetized using 1–1.5% of isofluorane, mixed with oxygen, and placed in a small animal stereotaxic frame (Kopf Instruments, CA). After making a small incision to expose the scalp, a bone scraper was used to clean the skull above bregma and lambda and a dental drill (Osada, EXL-M40) was used to make a small craniotomy above the MFB. 6-OHDA (free base) was dissolved in saline (0.9%, w/v, NaCl with 0.1%, w/v, ascorbic acid), to a final concentration of 2.5 mg/ml, immediately before use to minimize the oxidative effects on 6-OHDA. A total volume of 1 μl of 6-OHDA (2.5 μg) or saline was injected at a rate ~200 nl/min into the MFB (at AP −0.7 mm and ML 1.1 mm from bregma, and DV 4.8 mm from the exposed dura surface). Injections were performed using a micropipette (VWR) pulled with a long narrow tip (diameter = 6–9 μm) using a micropipette puller (Sutter Instrument Co.). Micropipettes were calibrated to inject 90 nl of liquid for every 1 mm, as modified from [38]. Following surgery, the scalp was sutured and topical antibacterial ointment (Topazone) was applied. Analgesic (meloxicam: 1 mg/kg, i.p.) was administered after the surgery and daily until animal's recovered based on grooming and overall appearance. Mice were kept continuously on a heating pad (~35 °C), given daily injections of saline (500 μl, s.c.), and fed a dietary food supplement Pediasure (*ad libitum*) until they returned to their pre-surgery weight. The post-operative mortality rate was 14.7%.

2.3. Behavior

When using multiple tests on the same group of animals, the behavioral tests were performed in the same order for both experimental groups. For all non-automated tests, the behaviors were scored by an investigator who was blind to the treatment condition.

2.3.1. Limb-use asymmetry (cylinder) test

Mice were tested, under low light conditions, in the evening prior to the onset of the dark cycle. These testing conditions increased the overall movement and rearing of the mice in the cylinder and allowed us to obtain sufficient data during repeated testing, which otherwise led to habituation and a decrease in rearing (data not shown). The cylinder test was modified from the original paradigm used in rats [29] and behavioral impairment in this test was shown to be ameliorated following dopamine agonist treatment in 6-OHDA mice [39,40]. Animals were placed in a glass cylinder (8 cm diameter and 11 cm height for mice) and recorded for 5–10 min. The cylinder was placed next to a mirror in order to visualize limb use movements from all angles (Supplementary Videos 1a and 1b). Forelimb asymmetry was assessed by scoring independent, weight-bearing contacts on the cylinder wall of the ipsilateral (ipsi) or contralateral (contra) paw, relative to lesioned hemisphere, as well as movements made by both paws (both). The percentage of ipsilateral and contralateral touches, relative to the total number of touches (ipsi + contra + both = total), was calculated.

2.3.2. Automated treadmill gait test

Treadmill gait assessment was performed with the DigiGait imaging apparatus (Mouse Specifics Inc.) [41]. Gait impairment was previously shown to be ameliorated by pharmacological dopamine treatment in MPTP treated mice [42]. Mice were placed on a motorized treadmill within a plexiglass compartment (~25 cm long and ~5 cm wide). Digital video images were acquired at a rate 80 frames per second by a camera mounted underneath the treadmill to visualize paw contacts on the treadmill belt. The treadmill was set at a fixed speed of 16 or 18 cm/sec at which most animals were able to move continuously (Supplementary Videos 2a and 2b) [41,43]. A plastic bumper, located near the rear of the mouse, was used to nudge the mice to encourage movement if they stopped running while the treadmill was moving. The videos were analyzed by the DigiGait software, which automatically identifies the paw footprints, and then manual alterations in the contrast of the images were made, if necessary, to properly distinguish the footprints from the background. The images were then automatically processed by the software to calculate values for multiple gait parameters, including stride width, stride length, paw angle, and stride length variability (see Supplementary Fig. 3).

2.3.3. Manual gait analysis

Manual gait analysis was performed using a limb painting procedure similar to previous studies [44–47]. Gait impairment was shown to be ameliorated by dopamine agonist treatment in MPTP mice [42]. Mice were first trained to traverse a horizontal corridor leading directly into their home cage by gentle nudges in the appropriate direction if they stopped or attempted to turn around. After training, mice were injected with 6-OHDA or saline and tested at 3 weeks after surgery. The bottoms of their hindlimbs were painted, by brushing with non-toxic kids paint (Crayola LLC), and the mice were allowed to walk the path to their homecage on a piece of paper (Supplementary Videos 3a and 3b). The stride length was determined by measuring the distance between the same points, on the ball mount region of the footprint, in two consecutive footprints. Stride length was calculated from 2 to 3 hindpaw strides when the animal was walking continuously at a constant pace. Steps just before the entry to the homecage were not included since mice often slowed down and made smaller steps at this point. Median data from 4 to 6 strides across the two trials were calculated.

2.3.4. Challenging beam test

The challenging beam test was performed according to previous studies in Parkinsonian genetic mouse models [46,48–52] and impairment in the beam test was reversed following dopamine agonist treatment in a Parkinsonian genetic mouse model [50]. The beam, made of Plexiglas (Plastics Zone Inc., Woodland Hills, CA), consisted of four sections (25 cm each) of decreasing width (3.5 cm to 0.5 cm at 1 cm decrements), with an underhanging ledge (1 cm width) that was placed 1.0 cm below the upper surface of the beam. Two days of training were performed prior to the surgery. For post-surgical testing (at 3 weeks after surgery), a mesh grid (1 cm^2) of corresponding width was placed over the beam surface, leaving approximately 1 cm space between the grid and the beam surface. Animals were then videotaped while traversing the grid-surfaced beam for a total of five trials after the surgeries (Supplementary Videos 4a and 4b).

Videotapes were viewed and scored in slow motion for the number of errors, number of steps, and time to traverse the beam across five trials. An error was counted when, during a forward movement, a limb (forelimb or hindlimb) slipped through the grid and was visible between the grid and the beam surface. Errors were not counted if the animal was not making a forward movement or when the animal's head was oriented to the left or right of the beam. Error per step scores, number of steps, and time to traverse the beam were calculated for saline and 6-OHDA-treated mice. Median data across the five trials were calculated.

2.3.5. Adhesive removal

The adhesive removal test was adapted from studies in rats [29,53] and in genetic PD mouse models [46,48,49,51]. Prior to surgery, two training trials were performed by placing adhesive dots (0.6 cm diameter, Avery) on the plantar surface of both forelimbs simultaneously. Training was used to acclimate the mice to the sensory stimuli (adhesive dots), which decreases their anxiolytic responses during the testing sessions post-surgery. At 3 weeks after 6-OHDA or saline injections, adhesive dots were placed on both forelimbs and the time to remove the dot from each forelimb was recorded (Supplementary Videos 5a and 5b). If a mouse did not remove either or both stickers within 60 s, the animal received a score of 60 s for the respective forelimb(s). Median data were calculated across three trials.

2.3.6. Adjusting steps (stepping) test

The adjusting steps test was adapted from studies in rats [54–57] and MPTP-treated mice [58] and impairment in this test was reversed by pharmacological replacement of dopamine in MPTP mice [58]. Mice were held by the base of the tail with their hindlimbs suspended above the table and moved backwards at a steady rate so that they traversed 1 meter distance over about 3–4 s. The mice were video-recorded, during this movement, (Supplementary Videos 6a and 6b) and the video was analyzed offline by counting the number of adjusting steps made with the contralateral or ipsilateral paw relative to the injected hemisphere over the total distance. Five trials were performed on mice at 3 weeks after surgery and median data were calculated across the trials.

2.3.7. Pole test

The pole test has been used to detect bradykinesia and motor coordination in PD mice [46,48,50,59–61]. Behavioral deficits in the pole test have been ameliorated by dopamine agonist treatment in MPTP mice [59,61] and in a Parkinsonian genetic mouse model [50]. The mice were placed facing upwards at the top of a wooden pole (50 cm long and 1 cm in diameter) that led into their home cage. The mice were trained, in two sessions on consecutive days before 6-OHDA or saline injections, to