Published in final edited form as:

J Neuroimmune Pharmacol. 2012 March ; 7(1): 279–288. doi:10.1007/s11481-011-9269-4.

Murine Motor and Behavior Functional Evaluations for Acute 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) Intoxication

Jessica A. L. Hutter-Saunders,

Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198, USA

Howard E. Gendelman, and

Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198, USA

University of Nebraska Medical Center, 985930 Nebraska Medical Center, Omaha, NE 68198-5930, USA, hegendel@unmc.edu

R. Lee Mosley

Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198, USA

Abstract

Acute intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces nigrostriatal neurodegeneration that reflects Parkinson's disease (PD) pathobiology. The model is commonly used for rodent studies of PD pathogenesis and diagnostics and for developmental therapeutics. However, tests of motor function in MPTP-intoxicated mice have yielded mixed results. This unmet need reflects, in part, lesion severity, animal variability, and the overall test sensitivity and specificity. In attempts to standardize rodent motor function and behavioral tests, mice were trained on the rotarod or habituated in an open field test chamber, and baseline performance measurements were collected prior to MPTP intoxication. One week following MPTP intoxication, motor function and behavior were assessed and baseline measurements applied to post-MPTP measurements with normalization to PBS controls. Rotarod and open field tests assessed in this manner demonstrated significant differences between MPTP- and saline-treated mice, while tests of neuromuscular strength and endurance did not. We conclude that the rotarod and open field tests provide reliable measures of motor function for MPTP-intoxicated mice.

Keywords

Parkinson's disease; MPTP; Behavior; Mice; Rotarod; Open field; Grooming

Introduction

Parkinson's disease (PD) is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) and decreased levels of dopamine in the

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Correspondence to: Howard E. Gendelman.

Electronic supplementary material The online version of this article (doi:10.1007/s11481-011-9269-4) contains supplementary material, which is available to authorized users.

Disclosures The authors have no financial conflict of interest.

putamen of the dorsolateral striatum (Bernheimer et al. 1973). The loss of dopamine in the striatum manifests clinically as motor disabilities characteristic of Parkinsonism; these include bradykinesia, resting tremor, muscular rigidity, and gait disturbances. Disease diagnosis is based on motor symptoms, which are evident only after the loss of greater than 50% of SNc dopaminergic neurons and 60–80% of striatal dopamine (Bernheimer et al. 1973). Parkinsonism is ameliorated with L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor to dopamine, which increases dopamine levels in the striatum. While combinations of L-DOPA and carbidopa (a dopamine decarboxylase inhibitor) remain the gold standard for treatment, new therapeutics are being investigated. To this end, the need for translational PD animal models that enable the evaluation of both histopathological characteristics and motor dysfunction is significant.

The acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of nigrostriatal degeneration recapitulates dopaminergic neuron loss seen in PD. It is currently the most commonly used toxin-induced mouse model of PD (Jackson-Lewis and Przedborski 2007). Nonetheless, while the acute MPTP intoxication induces significant degeneration of nigral dopaminergic neurons and loss of striatal dopamine, it does not produce Lewy body inclusions, a typical histopathological feature of idiopathic PD. Furthermore, acute MPTP-intoxicated mice show only subtle motor function deficits, which are difficult to measure (Meredith and Kang 2006). Indeed, past studies of locomotion in the acute MPTP mouse model yielded conflicting results (Sundstrom et al. 1990; Fredriksson and Archer 1994; Schwarting et al. 1999; Sedelis et al. 2000; Tillerson and Miller 2003; Meredith and Kang 2006; Keshet et al. 2007; Petzinger et al. 2007; Hirst and Ferger 2008; Meredith et al. 2008; Luchtman et al. 2009). Such incongruous results suggest that confounders of behavior and motor function testing may include the dose of MPTP, the method of MPTP administration, lack of lesion severity, poor correlations between loss of neurons and striatal termini, animal variability, and overall sensitivity and specificity of tests (Rousselet et al. 2003).

Prior studies demonstrated decreased motor function and abnormal behaviors of MPTP-intoxicated mice at hours to days following intoxication (Schwarting et al. 1999; Sedelis et al. 2000; Hirst and Ferger 2008; Luchtman et al. 2009). However, whether this immediate deficit reflects nigrostriatal degeneration per se is unlikely. Systemic intoxication with MPTP induces generalized narcosis leading to alterations in normal motor function, balance, grooming behaviors, eating and drinking, and causes hypothermia; all of which may be evident for several days after intoxication (Banerjee et al. 2008). Thus, the changes in behavior and motor function seen by others (Schwarting et al. 1999; Sedelis et al. 2000; Hirst and Ferger 2008; Luchtman et al. 2009) could reflect the systemic effects of MPTP and may not serve as a direct measure of nigrostriatal degeneration. Thus, the goal of the current study was to develop reproducible means to assess motor function and behaviors that occur as a consequence of dopaminergic neuron loss induced by acute MPTP intoxication.

Materials and methods

Animals

Adult male C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice 10–18 weeks of age were housed four or five to a cage, maintained on a 12:12 h light/dark cycle with ad libitum access to food and water, and were randomly assigned to treatment groups before motor function and behavior training. For each experiment, mice used were of the same age. The study was conducted in accordance with the animal care guidelines issued by the National Institutes of Health and approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center.

Acute MPTP intoxication

Mice received four subcutaneous injections, one every 2 or 3 h of either vehicle (phosphate buffered saline (PBS) at 10 ml/kg) or MPTP-HCL (18, 20, 22, or 24 mg/kg, free base in PBS; Sigma-Aldrich Co, St. Louis, MO). MPTP handling and safety measures were in accordance with National Institutes of Health, the University of Nebraska Medical Center, and prior published guidelines (Przedborski et al. 2001).

Immunohistochemistry

One week after MPTP or PBS administration, mice were terminally anesthetized with pentobarbital and transcardially perfused with PBS then 4% paraformaldehyde (PFA, Sigma-Aldrich Co) in PBS. Brains were harvested and post-fixed in PFA/PBS overnight, cryoprotected in 30% sucrose in PBS for 2 days, snap frozen in 2-methylbutane, embedded in OCT compound (Sakura Finetek USA, Inc., Torrance, CA), and 30 μ m sections were collected from the midbrain and basal ganglia using a cryostat (CM1900, Leica, Nussloch, Germany). Sections were processed free-floating in 48-well plates. Tissue sections were probed using rabbit anti-tyrosine hydroxylase (TH) as the primary antibody (1:2,000 for substantia nigra, 1:1,000 striatum, Calbiochem, EMD Chemicals, Gibbstown, NJ) and biotinylated goat anti-rabbit IgG as the secondary antibody (1:400, Vector Laboratories, Inc., Burlingame, CA). Brain tissue sections were incubated in streptavidin–horseradish peroxidase (HRP) solution (ABC Elite vector kit, Vector Laboratories, Inc). Immunostaining for TH expression was visualized using diaminobenzidine (Sigma-Aldrich Co) as the chromagen and mounted on slides with thionin counterstain for Nissl substance. Neurons expressing TH and containing Nissl-stained nucleus were considered dopaminergic. Photomicrographs were taken with a Nikon TE300 microscope. The contrast of photomicrographs was adjusted in Adobe Photoshop CS3 Extended version 10.0.1 using the Auto Contrast function.

Quantification of neuronal survival

TH- and Nissl-stained neurons in the substantia nigra were counted by stereological analysis in a blinded fashion with StereoInvestigator (MicroBrightField, Inc., Williston, VT), using the Fractionator probe module as previously described (Benner et al. 2004). The densities of dopaminergic neuron axonal termini in the striatum were determined by measuring the quantitative densitometric analysis of TH-stained termini as previously described (Benner et al. 2004).

Motor function and behavior tests

Forced motor function was measured using both the traditional constant speed rotarod and the accelerating rotarod methods. Neuromuscular function and strength were measured using the paw grip endurance test (also known as the “wire hang test”) and grip force recordings. Gate was analyzed with the ink/paw prints test. Natural behavior and movement were measured using the open field test. All tests were performed during the day in ambient light except for the open field testing, which was conducted during the day in dim light. Here, all tests are briefly described in their most commonly cited form, as well as with modifications (LeDoux 2005; Meredith and Kang 2006; Crawley 2007).

Constant speed and accelerating rotarod tests

For the traditional constant speed rotarod test, mice were trained and tested as previously described with slight modifications (Rozas et al. 1997; Rozas et al. 1998). Mice were trained and tested at several different speeds (measured in rpm), and the overall rod performance was calculated as the latency to fall from the rotating rod. In early experiments, mice were trained to balance and move on a rotating rod, 12.5 cm in circumference (mouse size), for

three consecutive days. Mice were conditioned at five speeds (5, 10, 15, 20, and 25 rpm) for 60 s per speed with at least 5 min rest between each speed. Mice were tested for motor deficit on the rotarod 6 and 7 days after PBS or MPTP treatment at 14, 16, 18, 20, 22, 24, and 26 rpm for 150 s at each speed with 5 min rest between trials. The overall rotarod performance was calculated as the area under the curve as a function of latency to fall from the rod versus rpm. The latency to fall was the mean of the two values obtained from testing on the two consecutive days (Rozas et al. 1997). In later experiments, mice were trained on a rod 22.5 cm in circumference (rat size) at 5 and 10 rpm for 5 min at each speed with at least 5 min rest between each speed. To record a baseline of motor function for each mouse, mice were tested 24 h after training was complete and 24 h before PBS or MPTP treatment. Mice were tested at four speeds (4, 6, 8, and 10 rpm). Testing consisted of three trials per speed (90 s). Each individual mouse's performance score was determined by normalizing the mean of the three trials at each speed to the baseline score. Mice were tested weekly beginning at 6 days after treatment.

A previous report suggested that the accelerating rotarod may be more sensitive to motor function deficits in MPTP-intoxicated mice than the traditional constant speed rotarod (Keshet et al. 2007). The accelerating rotarod differs from the traditional test as it measures the latency to fall off the rod, which is steadily accelerating in rpm over a defined period of time. Here, mice were tested starting 1 week after PBS or MPTP treatment. Mice were placed on the rod (12.5 cm in circumference) that was rotating at 5 rpm and the speed increased to 30 rpm or from 10 to 40 rpm over 5 min. Three trials were conducted per mouse with at least 5 min rest between trials. Each mouse was scored, in seconds, for the length of time on the rod, and the final performance score was an average of the three trials (Jones and Roberts 1968; Keshet et al. 2007).

For experiments using the mouse-sized rod, we used a Rotamex 4/8 System (Columbus Instruments, Columbus, OH, USA); for experiments using the rat-sized rod, we used the Accuroto Rotarod (AccuScan Instruments Inc., Columbus, OH, USA). No extreme negative reinforcement (e.g., electric shock) was used in training of mice; when mice fell off the rod during training, they were placed back on the rod until the training session was completed.

Paw grip endurance and grip strength

The paw grip endurance (PaGE) method and the grip force-recording test are often used as measures of motor strength. PaGE is designed to assess the grip strength of mice, which may be indicative of a decrease in motor skills and was performed here as described by Weydt et al. (2003). Briefly, each mouse was placed on a wire lid from a conventional rodent housing cage; the lid was gently shaken to induce gripping and turned upside down (180°). The latency until the mouse released both hind limbs was measured in seconds. Each mouse was tested three times with an arbitrary maximum of 90 s, and the longest latency to fall or release both hind limbs was recorded (Weydt et al. 2003).

The grip force-recording test measures forelimb muscle strength; however, this test uses a dynamometer to measure force and is considered to be a more objective assessment than PaGE. To measure grip strength, we used a digital force gauge by Chatillon mounted on a grip strength platform with a "front grip" attached to the dynamometer (San Diego Instruments, San Diego, CA 92126). The force scale was positioned horizontally, and mice were individually lowered by the tail toward the apparatus until they grasped the front grip with their forelimbs. The mouse was then steadily pulled backward in a horizontal plane. The force applied to the grip immediately before release of the grip is recorded as the "peak tension" (AMETEK TCI Division, Chatillon Force Measurement Systems, Largo, FL). The test was repeated three consecutive times per mouse 1 week after MPTP intoxication, and

the measurement of strength (peak tension) for each mouse was an average of the three trials.

Ink paw print test

The ink paw print test is designed to assess the walking pattern of mice. To measure stride length, we conducted the ink paw print test as previously described (Crawley and Paylor 1997). Waterproof ink was applied to the feet of the individual mice, and they were placed on a paper-lined gangway and allowed to walk to the end and into their home cage. The total length of the gangway was approximately 20×4 in. with a 3-in. wall on either side. Prints recorded in the middle 11 in. of the gangway were analyzed. The average stride length was determined by measuring the distance from the center of one paw print to the next on the same side of the body. All recorded stride lengths per mouse were averaged to attain a mean stride length per mouse.

Open field activity test

Mice suffering from neurological impairments may not move normally or explore the open field test chamber whether due to motor or cognitive deficits (Hall 1934). A Tru Scan 2.0 by Coulbourn Instruments (Whitehall, PA 18052) was used to conduct open field testing, which measures many types of unforced movement such as total distance traveled, average speed of movements, time spent in specific areas, rearing, and stereotypic behaviors. Movements of each mouse were automatically recorded and measured within a standard arena by recording breaks in photo beams that are spaced at 2.54 cm apart and span the arena at two levels, floor and vertical planes, with a spatial resolution of 1.27 cm. The arena used here measured 40.64×40.64×40.64 cm. A lid was placed on the top of the chamber to reduce noise and light entering the chamber. Mice were habituated to the chamber for 10 min per day for three consecutive days. Twenty-four to 48 h after habituation was complete, a baseline level of movements was recorded over a 25-min period during a pre-treatment test. Twenty-four hours after the pre-treatment test, mice were treated with PBS or MPTP. Mice were then tested weekly starting at 1 week post-treatment. As defined by the manufacturer, stereotypic moves are repetitive movements that start and return to the original position in less than 2 s with at least three of such movements initiating a stereotypy episode. Movements that change less than ± 0.999 beam coordinates are denoted as “type 1” stereotypy moves, while “type 2” stereotypy moves are movements that change less than ± 1.499 beam coordinates. Due to the stereotypic nature of the syntactic grooming chain, and non-syntactic grooming, these behaviors are measured under stereotypy type 1 and/or 2. However, the system does not distinguish between syntactic and non-syntactic grooming. Syntactic grooming consists of serially order movements of four phases (elliptical strokes, unilateral strokes, bilateral strokes, and flank licking) that are governed by the rules of action syntax (Berridge et al. 1987). The chain has a mathematical predictability and occurs with a frequency that is over 13,000 times greater than expected by chance (Berridge et al. 1987). This behavior is implemented by the dorsolateral striatum (Aldridge and Berridge 1998) and is an area of the basal ganglia affected by dopamine levels (Berridge et al. 2005; Taylor et al. 2010). Thus MPTP-mediated depletion of dopamine in this area should interrupt syntactic grooming behavior and be of use in analysis of neuronal loss. Each motor action can also occur alone and out of sequence (called non-syntactic grooming), yet these behaviors are also governed by neurons of the dorsolateral striatum (Aldridge et al. 2004), and they may be similarly affected by MPTP-mediated dopamine depletion. A subset of PBS-treated mice ($n=3$) and MPTP-intoxicated (18 mg/kg/injections MPTP) mice ($n=3$) were videotaped and visually scored for syntactic grooming sequences over 10 min in a blinded fashion.

Statistical analysis

Data are expressed as the mean±standard error of the mean. Statistical significance was evaluated by applying a Student's *t* test or a one-way ANOVA followed by post hoc paired comparisons using Tukey's honest significance or Fisher's LSD test. Data was considered significant at the *p* 0.05 level. In later experiments, mouse behavior and motor function were analyzed by comparing post-MPTP intoxication test results to test results collected before MPTP intoxication on a mouse-to-mouse basis. All statistical tests were carried out using GraphPad Prism 4.0c for Mac OS X (GraphPad Software Inc., San Diego, CA) or Statistica (StatSoft, Inc., Tulsa, OK).

Results

Measureable dopaminergic neuron loss by MPTP intoxication without behavior and motor function deficits

A broad range of motor functions and behaviors are under the control of dopamine-mediated circuitry of the basal ganglia, some of which are affected by MPTP intoxication (Fig. 1a). One week after acute MPTP intoxication at a dose of 18 mg/kg/injection, significant decreases in the number of dopaminergic neurons in the SNc and their projections to the caudate putamen (CPu) of the dorsolateral striatum were visualized by immunohistological staining for expression of tyrosine hydroxylase (TH; Fig. 1b). Additionally by stereological analysis, MPTP treatment at 18 mg/kg/injection produced a mean TH neuron loss of 52% in the SNc (Fig. 1c) and a 66% decrease in TH striatal termini compared to PBS-treated mice (Fig. 1d). The neuropathology induced by acute MPTP intoxication suggested deficits in motor function and behavior under the control of the basal ganglia should be measurable. Thus, we assessed behavior and motor functions in MPTP-intoxicated mice.

For the traditional constant speed rotarod test, mice were trained and tested at several different speeds, and the overall rod performance was calculated as the latency to fall from the rotating rod (Rozas et al. 1997; Rozas et al. 1998). We initially trained and tested mice on a "mouse-sized" rotating rod of 12.5 cm circumference. Twenty-four hours after training was complete, mice were injected with either PBS or MPTP at 18 mg/kg/injection. Six days after injections, mice were tested for motor deficits. No significant differences were detected in the rotarod performances of the PBS- and MPTP-injected mice (Fig. 1e).

Previous works suggested that motor function deficits in MPTP-treated mice can be detected using the accelerating rotarod method (Keshet et al. 2007). However, at 8 days post-MPTP intoxication, no significant differences in time on the rod were discerned between PBS- and MPTP-intoxicated mice when the rod accelerated from 5 to 30 rpm over 5 min (Fig. 1f). The mice were also tested with the rotation speed increasing from 10 to 40 rpm over 5 min. No significant differences were detected between groups (*p*>0.05, data not shown). Together, the lack of differential results from both the traditional rotarod test and the accelerating rotarod test led to assessments of MPTP dose effects on motor function.

Mouse rearings diminish with increased MPTP dose

Past studies have shown that the degree of dopamine and dopaminergic neuron loss is dependent on the dose of MPTP (Heikkila et al. 1984; Sonsalla and Heikkila 1986; Seniuk et al. 1990). Here, we investigated the effect of MPTP dose on behavior. Mice were injected with PBS or MPTP (18, 20, 22, or 24 mg/kg×4 injections) with one injection every 3 h (Keshet et al. 2007). Mice were tested for motor deficits and behavior alterations at 1 and 2 weeks post-intoxication, respectively. Accelerating rotarod performance, PaGE performance, grip strength, and stride length were assessed at day 6 or 7, while open field activity was assessed at day 14 (Fig. 2). For the accelerating rotarod test, each mouse was

tested three times in one session with a 57-min resting period between each trial. The rotarod (12.5 cm circumference) was set to accelerate from 10 to 40 rpm over a 5-min period. Each mouse was scored for mean latency to fall. No significant differences were discernible between any of the groups (Fig. 2a). The PaGE test assesses motor functions under supraspinal control such as skilled forepaw use (Meredith and Kang 2006), which is sensitive to striatal dopamine losses (Tillerson et al. 2002), yet no significant differences were found (data not shown). We reasoned that the criteria of either both hind limbs or all four limbs releasing their grip (Weydt et al. 2003) were not sensitive measures of nigrostriatal degeneration. Thus, mean grip strength of each mouse was recorded using a digital force gauge, but no significant differences between groups were detected (Fig. 2b). Differences in gait measured as a function of stride length (supplemental Fig. 1) at 7 days post-treatment were not detected (Fig. 2c). The open field test was conducted at 14 days post-MPTP injection. While no significant differences in rearing were discerned between the groups, most likely due to coefficient of variances within groups ranging from 34% to 67%, analysis of variance for linear trends revealed a significant diminution in the number of rearings with increasing MPTP dose ($p=0.026$, $R^2=0.142$, Fig. 2d).

Normalization of behavior scores to pre-treatment baseline diminishes variability

We next modified the analysis of the traditional rotarod test. We trained the mice as described, but conducted a pre-treatment test 24 h before MPTP injection to use as a baseline of performance for each individual animal. We also used a larger circumference rod (22.5 cm) to increase difficulty and better provide a direct measure of supraspinal function, as it requires skilled use of digits to stay on the rotating rod. Forty-eight hours after the pre-treatment test, mice were injected with PBS or MPTP at dose rates of 18 or 20 mg/kg/injection. Higher doses of MPTP (22 and 24 mg/kg/injection) were excluded from this experiment due to equivalent or greater loss of dopaminergic neurons at lower doses and increased mortality associated with the higher doses. Performance among mice increasingly varied with speed of the test even after extensive training and regardless of pre-treatment group (Fig. 3a). Indeed, the coefficient of variances of latencies for mice tested at 4, 6, 8, and 10 rpm prior to MPTP were 5%, 21%, 33%, and 50%, respectively, showing a progressive increase in variances with speed. Thus, we reasoned that the intra-group variation might decrease statistical power and confound statistical comparisons between PBS- and MPTP-treated mice. Without normalization, the overall coefficient of variances for rotarod latencies of PBS- and MPTP-treated groups progressively increased (5%, 17%, 25%, and 44%) with increasing speed (4, 6, 8, and 10 rpm), and significant differences were not realized between any treatment groups at 1 week (Fig. 3b) or 2–3 weeks (data not shown) post-MPTP intoxication, although trends of diminished rotarod performance were evident with increasing speed. Moreover, retrospective power analysis indicated low power (<0.36) among all groups on day 8 post-treatment, again due in part to increased within-group variances. We reasoned that by normalizing individual post-test performances with each pre-treatment test performance we could diminish intra-animal variation and increase statistical power. Furthermore, to counter variances associated with different speeds and better facilitate comparisons, all groups were normalized to the PBS control group as a relative comparator (Fig. 3c). Similar to non-normalized data, significant differences in rotarod performances at 4, 6, and 8 rpm were not detected in MPTP-treated groups compared with PBS controls, although trends of diminished function were evident. Most notably, normalization of performance data at 10 rpm showed significant diminution of relative latencies to fall from the rotarod in mice 1 week after MPTP intoxication with either 18 or 20 mg/kg/injection ($p<0.001$ and $p<0.01$, respectively) compared with PBS controls. Similar results were observed at 2 and 3 weeks post-MPTP intoxication when rotarod performances at 10 rpm were still diminished in mice treated with MPTP at 18 or 20 mg/kg/injection ($p<0.01$ and $p<0.05$, respectively, and data not shown). As expected, retrospective

analysis of week 1 post-treatment performances at 10 rpm showed that the power for between-group effects was substantially increased to 0.70 for normalized data compared with 0.24 for non-normalized rotarod performances.

In rodents, dopamine plays a crucial role in implementation of repetitive movements, such as sequential grooming, as demonstrated by impaired loss of these behaviors with ablation of dopaminergic neurons and interruption of the dopamine circuitry (Berridge 1989; Cromwell and Berridge 1996; Aldridge and Berridge 1998; Berridge et al. 2005). We assessed repetitive motor functions using open field modules that measure stereotypic movements, which include grooming patterns, but do not discriminate between syntactic grooming and non-syntactic stereotypic movements. We found that MPTP intoxication, by 1 week post-treatment, diminished the relative number of stereotypic type 1 moves compared with PBS-treated mice ($p < 0.001$, Fig. 4a). Moreover, stereotypic type 2 moves in MPTP-intoxicated mice were also reduced by 1 week post-injection ($p < 0.01$, Fig. 4b). In support of this data, the number of completed syntactic grooming chains in MPTP-intoxicated mice trended below those observed in PBS-injected controls ($p = 0.15$, Supplemental Fig. 2), while the low number of syntactic grooming movements supports the notion that this behavior does not comprise all stereotypy behavior, in toto. Representative examples of syntactic grooming by a PBS-injected control mouse and an MPTP-intoxicated mouse can be viewed in the supplemental materials, video 1.

Discussion

The development of reproducible mouse-specific behavioral measurements of nigrostriatal neurodegeneration is warranted by the lack of reproducible tests of motor function deficit in MPTP-treated mice (Dauer and Przedborski 2003). While most human clinical symptoms may not be apparent in MPTP mice, we have shown that mouse motor functions under the influence of the basal ganglia, such as stereotypic movements, may be used as a measure of nigrostriatal integrity. Movement disorders arise with disturbances to the structures of the basal ganglia: the substantia nigra (SN), striatum, internal and external segments of the globus pallidus, and subthalamic nuclei (Jurkowski and Stacy 2005). The SN is composed of the pars reticulata and the pars compacta, the latter being the main afferent compartment of dopaminergic innervation to the caudate putamen of the dorsal striatum. Damage to the SN pars compacta or caudate putamen or a loss of neurotransmitter signaling to the striatum causes noticeable deficits in motor functions that require fine forepaw movements and digits to work independently, like walking on a rough terrain and grooming. Thus, the MPTP-induced nigrostriatal degeneration and subsequent decrease of dopamine in the striatum should induce loss of selective motor functions. However, past studies using motor function tests as a measure of nigrostriatal damage yielded conflicting results (Sundstrom et al. 1990; Fredriksson and Archer 1994; Schwarting et al. 1999; Sedelis et al. 2000; Tillerson and Miller 2003; Meredith and Kang 2006; Keshet et al. 2007; Petzinger et al. 2007; Hirst and Ferger 2008; Meredith et al. 2008; Luchtman et al. 2009), suggesting that motor function assessment cannot be successfully conducted on mice acutely intoxicated with MPTP. Indeed, results of the current study that failed to reach statistical significance suggest that methods simply measuring gross motor functions, such as the ink paw print or grip strength tests may not provide the sensitivity needed to detect a motor deficit in fine forepaw use in mice acutely intoxicated with MPTP.

The rotarod is widely used as a test of motor function in rodents. Here, we found that when using a “mouse-sized” rod, mice are able to grip the rod easily and often rotate with the rod, confounding assessment of motor function, while the use of a larger circumference “rat-sized” rod seemed to increase difficulty of the test as demonstrated for saline-injected mice, which exhibited diminished latencies to fall on the larger circumference rod. It is important

to clarify that the apparent increase in difficulty was not due to a change in the speed at which the mice were walking on the rod. The change in rpm between the “mouse-sized” and “rat-sized rods” is due to the difference in circumference of the rods and the actual walking speed (i.e., kilometers per hour) did not change. For example, on the 12.5 cm in circumference “mouse-sized” rod, 18 rpm is equivalent to 0.135 kph, while 10 rpm on the 22.5 cm in circumference “rat-sized” rod is equivalent to 0.135 kph. Thus, while the rpm varied, the actual speeds at which the mice were walking were comparable. The apparent increased difficulty of the rat-sized rod may be due to the change in the slope of the surface on which the mice are walking or slight differences in the texture of the surface of the rods. In spite of these improvements, large variances rendered analysis of non-normalized rotarod performance to non-significant levels due to low power (0.24), and thus, would require increased numbers of animals to attain significance. For these data, prospective analysis indicated that over 100 animals would be required to attain power levels of 0.80 or above. In contrast, normalization of data diminished variances and increased power (0.70), thus lowering the required sample size to 30 animals to attain power levels of 0.80.

Stereotypic behaviors measured by open field tests were of interest as damage to the basal ganglia's dopamine-mediated circuitry should be apparent in measures of syntactic grooming or other stereotypic movements. Others have shown that implementation of action syntax, such as syntactic grooming, is controlled by the dorsolateral striatum in rodents, and other areas of the basal ganglia are thought to play a role in non-syntactic grooming behaviors (Cromwell and Berridge 1996; Aldridge and Berridge 1998). Indeed, tests that targeted motor functions under the control of the basal ganglia, such as measuring stereotypic moves by open field testing, yielded statistically significant differences between MPTP-intoxicated and control mice, suggesting these tests are more sensitive in detecting nigrostriatal damage.

The pathology of the acute MPTP mouse model is induced by the subcutaneous or intra-peritoneal administration of MPTP in one or several injections within a 24-hour period. In contrast, administration of smaller MPTP doses, but given over several consecutive or non-consecutive days or weeks elicits a sub-acute or chronic intoxication with slower progression of subsequent dopaminergic neuronal loss (Jackson-Lewis and Przedborski 2007). In this study, the acute MPTP mouse model was used because of the time course and because it reproducibly depletes nigral dopaminergic neurons by 40–50% and striatal termini by 70–80% in C57BL/6J mice (Jackson-Lewis et al. 1995). Acute MPTP intoxication induces a generalized toxicosis manifesting as hypothermia and alterations in heart rate and blood pressure, loss of spontaneous motor activity and coordination, decreases in drinking, eating, and grooming behaviors (Przedborski et al. 2001; Banerjee et al. 2008). However, the rate of neuronal death peaks at 2 days following intoxication (Jackson-Lewis et al. 1995), and thus, these early effects are not due to neuronal death. To abate toxicosis as a confounder of motor function deficits, mice were tested 1 week after MPTP intoxication as opposed to earlier time points. The dose of MPTP directly affects lesion size (Jurkowski and Stacy 2005), and thus, may also affect the degree of motor deficit and thus the researcher's ability to measure the deficit. We showed that some behaviors were affected in a dose-dependent manner. Furthermore, while higher doses of MPTP (22 and 24 mg/kg/injection) increased the number of deaths, doses of 18 and 20 mg/kg/injection over a 6–9 h period consistently induced a measurable motor deficit without high mortality.

In conclusion, we demonstrated that motor function and behavior testing yield significant results with the acute MPTP mouse model. This is seen principally when the test used measured functions under the control of the basal ganglia. We also show the utility of normalizing data to individual mouse pretests to eliminate variations between mouse groups. Taken together, the tests described herein should be valuable additions in the testing

paradigms in assessing behavioral and motor functions as a consequence of progressive nigrostriatal degeneration or during development of PD therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Megan Willer and Alex Braun for help with data collection; Rebecca Banerjee, Adelina Holguin, and Kalipada Pahan for advice on experimental methods and design; and Stephen Bonasera for discussions that enhanced the depth of this work. This work is supported by NIH grants 2R01 NS034239, P20 RR15635, P30 AI42845, P01 DA028555, P20 RR15635, and 5R01 NS36126, 1R01 NS070190, 1 P01 NS043985-01, P01 MH64570, 1R01 MH083516, P20 DA026146, and P01 NS31492.

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Fig. 1.

Acute MPTP intoxication damages the basal ganglia, which control motor function and action syntax, but the overall rod performance and the accelerating rotarod performance are not affected in C57BL/6J mice, 1 week after intoxication with 18 mg/kg/injection of MPTP. (a) Schematic of the mouse brain illustrating basal ganglia circuitry. Dopaminergic neurons within the SNc provide dopamine to the terminal fibers and synapses of the CPu. The CPu in turn contains efferent connections to the thalamus and receives afferent connections from the cerebral cortex. (b) Photomicrographs of the SN and striatum of representative mice injected with PBS (*left panels*) or MPTP (18 mg/kg/injection × 4 injections) (*right panels*); the latter shows loss of SN dopaminergic neurons and their striatal projections. Scale bar is 500 μm in SN; striatum were taken at 200× magnification. (c, d) Coronal sections of ventral midbrain and striatum were immunostained for TH. (c) Dopaminergic neurons in the SNpc were identified as TH+ Nissl+ neurons (*black bars*), while non-dopaminergic neurons were identified as TH- Nissl+ neurons (*gray bars*). (d) Mean densities of striatal dopaminergic termini were determined by digital image analysis. Application and performance analysis of rotarod with (e) discontinuous advancing speed or (f) continuous accelerating speed for mice administered PBS or MPTP (18 mg/kg/injection) and tested for latency to fall from rotarod at (e) 6 or (f) 8 days post-injection. Bar graphs represent means ± SEM (c–e) and means (*horizontal lines*) among the data points (f) for eight animals/group. Significant differences between means were determined by Student's *t* test where ^a*p* 0.05

Fig. 2.

Motor function measurements of mice treated with graded doses of MPTP. C57BL/6J mice were treated with PBS or four injections of MPTP at 18, 20, 22, and 24 mg/kg/injection. One week after treatment mice were assessed for latency to fall in accelerating rotarod tests (a), front paw grip strength (b), stride length (c), and the number of rearing events in the open field test (d). Results are expressed as means (*horizontal lines*) among individual data points (a–c) and means ± SEM (d) for $n=5-10$ animals/group. Significant differences between means were determined by one-way ANOVA, and no significant differences were found.

Fig. 3.

MPTP-induced motor function deficits determined by normalization of rotarod performance to pre-treatment test values. (a) Rotarod performance of unassigned mice was measured prior to MPTP or PBS treatment. Un-normalized (b) and normalized (c) rotarod performance of mice, 1 week after PBS treatment (*squares* and *white bars*), MPTP treatment at 18 mg/kg/injection (*triangles* and *gray bars*), or MPTP at 20 mg/kg/injection (*inverted triangles* and *black bars*). Latencies to fall from the rod are presented for eight to nine animals/group as individual values and means (*horizontal lines*) in the *left panels* of **a**, **b**, and **c**, or as means $\pm$ SEM for each speed tested in the *right panels*. Significant differences among means were determined by one-way ANOVA and pair-wise comparisons determined by Tukey's HSD post hoc analysis where ^a p 0.05 compared to PBS

Fig. 4.

Basal ganglia-specific behavior deficits determined by normalization of post-treatment stereotypic movements to pre-treatment test values. Mice were evaluated in the open field test arena prior to treatment, and then treated with four injections of PBS or MPTP (18 or 20 mg/kg/injection). One week after treatment, mice were evaluated for type 1 (**a**) and type 2 (**b**) stereotypy movements by the open field test. Individual and means (*horizontal lines*) of stereotypic moves normalized to baseline pre-treatment test values and PBS controls were determined for eight to nine mice/group. Significant differences among means were determined by one-way ANOVA and pair-wise comparisons determined by Tukey's HSD post hoc analysis where ^a $p < 0.05$ compared to PBS. Mice treated with 20 mg/kg/injection of MPTP exhibited fewer type 1 ($p < 0.001$) and type 2 ($p < 0.01$) stereotypic movements compared to PBS-treated mice.