MPTP-Induced Models of Parkinson's Disease in Mice and Non-Human Primates

Since its discovery in 1983 (Langston et al., 1983), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been widely used to create animal models of Parkinson's disease (PD). MPTP, a pro-toxin, is converted by monoamine oxidase-B (MAO-B) to 1-methyl-4-phenylpyridinium ions (MPP^+). MPP^+ is selectively transported by the dopamine transporter (DAT) into dopaminergic neurons, where it exerts toxic effects. MPP^+ inhibits complex 1 of the mitochondrial electron transport chain, eventually causing the increased production of highly reactive oxygen species that, through reactions with cellular proteins, nucleic acids, lipids, and other molecules, cause substantial cellular damage precipitating cell death (Shastry, 2001). Thus, with appropriate dosing regimens, MPTP administration can produce a selective lesion of dopaminergic neurons of the nigrostriatal tract, replicating characteristics of the primary pathology of Parkinson's disease.

Several species, including humans, monkeys, and mice, but not rats, are sensitive to the neurotoxic effects of MPTP. MPTP is routinely used to induce a parkinsonian-like pathology in mice and monkeys, and these models are used to study the development of this condition and its treatment. While the mouse MPTP treatment regimens cause a significant loss of brain dopamine neurons, questions remain as to whether the mouse model displays a behavioral profile appropriate for pharmacological study (see Sedelis et al., 2001, for a review of behavioral phenotyping of the MPTP-treated mouse). In contrast, MPTP-induced destruction of dopamine systems in the marmoset and macaque brains produces symptomatology similar to that observed clinically. Furthermore, chronic treatment with dopamine-replacement therapy in MPTP-lesioned primates is associated with side effects that are virtually identical to those observed in humans. For example, administration of L-DOPA to MPTP-lesioned primates causes levodopa-induced dyskinesia similar to that observed in patients with Parkinson's disease. Given these characteristics, the MPTP-lesioned primate model of Parkinson's disease is an excellent system for assessing the therapeutic and side-effect potential of drugs for the treatment of Parkinson's disease.

This unit describes two protocols that create MPTP-induced lesions in mice (see Basic Protocol 1) and marmosets (see Basic Protocol 2). Basic Protocol 2 also describes the induction of levodopa-induced dyskinesia. If marmosets are used to assess anti-parkinsonian agents, using repetitive behavioral assessment (see Basic Protocol 3), stable parkinsonism must be induced following the administration of MPTP (typically 8 to 12 weeks post-MPTP).

NOTE: All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) or must conform to appropriate governmental regulations regarding the care and use of laboratory animals in scientific experiments.

CAUTION: MPTP is a highly toxic compound and should be handled with extreme caution.

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MPTP-INDUCED DOPAMINERGIC LESIONS IN MICE

The effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on the nigrostriatal dopaminergic system of the mouse have been shown to be dependent on age (Gupta et al., 1986; Ricaurte et al., 1987), gender (Freyaldenhoven et al., 1996), strain and supplier (Heikkila, 1985; Sundstrom et al., 1987; Giovanni et al., 1991), dose, route, and schedule of administration of MPTP (Fredriksson and Archer, 1994). Accordingly, all these factors must be considered prior to commencement of a study. The protocol described below employs a common strain of mouse and yields a consistent destruction of dopaminergic pathways. Using this approach, it is possible to produce animals experiencing a 75% reduction in tyrosine hydroxylase (TH)-positive neurons in the substantia nigra and a 90% depletion of striatal dopamine and dopamine transporters (DATs) (Jackson-Lewis et al., 1995; Kuhlmann and Guilarte, 1999). These animals can be used to define the molecular and neurochemical abnormalities associated with this condition, and can also be used as subjects for testing the effectiveness of potential neuroprotective and neurorestorative agents. Compounds can be administered prior to and/or alongside MPTP treatment to assess the ability to protect against cell death, or after MPTP administration, to assess the ability to restore dopaminergic function once it has been compromised.

There are many dosing schedules available, the employment of which is dependent on the nature of the study. The most commonly used administration schedule for primary screening of neuroprotective therapies is to administer the test agent concurrently with MPTP administration. However, more specific schedules can be applied subsequently to assess whether the effect of the test substance is neuroprotective or neurorestorative. Depending on the duration of action of the test substance, it might be administered only once daily or with each dose of MPTP. Alternatively, a test agent may be administered prior to each MPTP treatment to determine whether it influences neurotoxicity. Potential neurorestorative agents would not typically be administered until 9 to 15 days after MPTP treatment. Many factors impact the choice of dosing regimen. The most conservative approach to avoiding false-negative results is to employ all three.

Materials

C57BL/6 male mice (25 to 30 g, 8 weeks old; Harlan)
Standard rodent diet
MPTP hydrochloride (Sigma)
0.9% sterile saline
Test compound(s)
Reference compound (e.g., 10 mg/kg selegiline, an MAO-B inhibitor; Sigma)
Rodent cages
1-ml syringes
21- and 23-G needles
Parafilm
Transparent plastic container to store MPTP-filled syringes

1. At least 7 days prior to the start of the experiment, house four mice/cage under a standard light/dark cycle (lights on: 0700 to 1900) with food and water available ad libitum.

   The animals should weigh 25 to 30 g at the start of MPTP administration. The experimental room should be designated for the use of MPTP (see Critical Parameters).

   The protocol described here minimizes variability, but it is recommended that group sizes of 12 to 14 be employed. Thus, in a typical study (e.g., three doses, one reference compound, one control group), ~60 mice would be used. These high n numbers per group will allow for anticipated morbidity and variance.

2. Dissolve MPTP hydrochloride in 0.9% saline to a final concentration of 200 mg/ml. Prepare sufficient MPTP for the whole experiment to minimize human exposure.

   CAUTION: MPTP is a highly toxic compound and should be handled with extreme caution. Wear personal protective equipment (PPE) required for the safe use of MPTP: a disposable, one-piece Tyvek suit with elasticized cuffs and hood; non-absorbent boots; a full-face respirator with disposable HEPA filter cartridges; and double-layered nitrile gloves (see Przedborski et al., 2001, for a detailed review of the safe use of MPTP).

3. Fill 1-ml syringes attached to 23-G needles with ~0.4 ml of 200 mg/ml MPTP solution. Cap the needles, wrap syringes in Parafilm, and store in a transparent plastic container labeled "MPTP-Neurotoxin" along with the user's name, contact details, and date. Store the container up to 2 months at –80°C until needed.

4. On day 1, 1 hr prior to each injection, remove the appropriate number of syringes (1 per animal) from the freezer and allow to thaw at room temperature in a fume hood.

   Each animal will receive a total of four injections of MPTP hydrochloride (20 mg/kg, i.p.) on day 1 at 1000, 1200, 1400, and 1600.

5. Weigh all animals, accurate to 1 g.

6. Immediately prior to each injection and in a fumehood, remove Parafilm wrap from syringe. Eject excess MPTP in syringe so the remainder will allow injection of 10 ml/kg, i.e., 20 mg/kg.

7. Inject MPTP intraperitoneally in a fumehood.

   It is typical to include an unlesioned control group in which no degeneration of the nigrostriatal tract would be anticipated. Such a control group would typically receive i.p. injections of 0.9% saline on day 1 at times equivalent to the MPTP group.

8. Return mouse to cage. Provide normal food and water for 8 days. Weigh animals daily.

   If the body weight of the animal drops more than 20% of what it was at the start of the experiment, sacrifice the animal. It would be expected that some animals may become hypolocomotor.

9a. On day 8, sacrifice animals by CO_2 asphyxiation in an airtight cage.

9b. When a test agent is being assessed for its ability to restore lost dopaminergic transmission, extend the protocol beyond day 15. Treat groups of animals as described in steps 1 to 8 to establish a lesion and then treat with the potential restorative therapy (typically for 7 to 21 days). Include groups that receive appropriate vehicle injections to control for animals that spontaneously recover.

10. Perform post-mortem assessment of the level of damage to the dopaminergic component of the nigrostriatal pathway.

    There are a variety of measures that can be made such as striatal tissue levels of dopamine and its major metabolites, e.g., dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), can be assessed using high-performance liquid chromatography (HPLC). Alternatively, striatal levels of the dopamine transporter (DAT) can be measured using immunohistochemical methods in tissue sections. Typically, cell death at the level of the substantia nigra pars compacta is assessed by tyrosine hydroxylase (TH) immunoreactivity carried out on tissue sections. The number of TH-positive cells can be estimated with the use of stereological techniques.

    Figure 5.42.1 illustrates the anticipated level of damage to the nigrostriatal dopaminergic system following administration of MPTP.

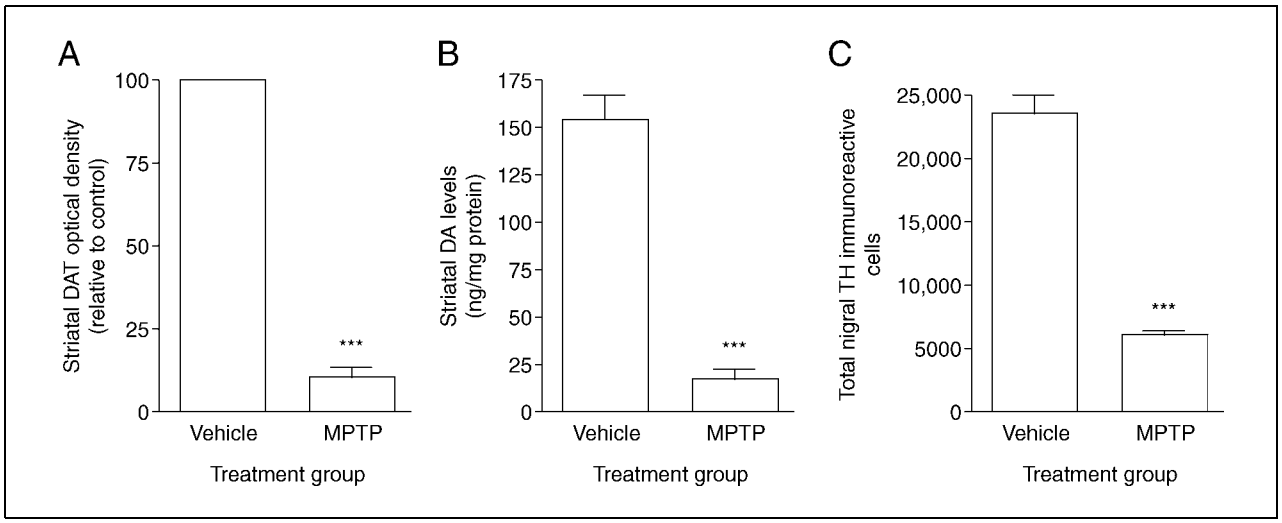

Figure 5.42.1 (**A**) Immunoblot analysis of dopamine transporter (DAT) protein following administration of vehicle or MPTP (20 mg/kg i.p., four times/day). For relative comparison, the results from the MPTP group were normalized against the vehicle group, which was set at 100%. (**B**) Striatal dopamine levels following administration of vehicle or MPTP (20 mg/kg i.p., four times/day). (**C**) The effect of administration of either vehicle or MPTP (20 mg/kg i.p., four times/day) on the number of TH-positive cells in the substantia nigra pars compacta, as assessed using an unbiased stereology counting technique. $n = 6$ per group. *** $p<0.001$ versus vehicle.

BASIC PROTOCOL 2

STABLE DOPAMINERGIC LESIONING AND PARKINSONISM IN MARMOSETS

This protocol allows for the production of a stable primate model of Parkinson's disease in which symptoms of the disorder remain stable over at least 6 months. This animal model is useful for postmortem studies to examine neuropathological aspects of the condition, and for testing new therapies for the treatment of this neurological condition. This model can also be used to produce animals exhibiting levodopa-induced dyskinesia (LID) following repeated treatment with levodopa; a side effect that compromises the utility of current anti-parkinsonian agents in patients.

Materials

Adult common marmosets (*Callithrix jaccus*), 300 to 400 g (Harlan)
Teklad Global 20% protein primate diet 2050 (Harlan Teklad)
MPTP hydrochloride (Sigma)
0.9% sterile saline
Test compound
Reference compound (e.g., L-DOPA, 10 to 20 mg/kg)

1-ml syringes
21- and 23-G needles
Parafilm
Transparent plastic container to store MPTP-filled syringes

NOTE: The room in which the marmosets are housed should be designated for the use of MPTP (see Critical Parameters).

1. House marmosets (ideally siblings) in pairs. Allow animals to acclimate to each other and the housing room for at least 2 weeks prior to commencing MPTP treatment. Provide free access to food and water. Rotate the fruit and food treat selection on a daily basis to provide novelty and encourage eating. Maintain the room at 25°C, 50% humidity on 12-hr dark/light cycles (lights on at 0800). During daylight hours,

provide changing audio and/or visual stimuli (e.g., radio or television) to enrich the environment. Action movies are recommended.

If using both male and female animals, house in same-sex pairs to avoid the possibility of pregnancy.

Although MPTP will produce an effective lesion in both male and female marmosets, females are generally preferred as they can be group housed without problems arising from fighting.

If animals are being used to produce tissue for post-mortem studies, it is necessary to include a control group. Such a control group should be of the same weight and sex as the "MPTP, parkinsonian" group and shoould receive 0.9% saline injections s.c. on days 1 to 5.

2. Dissolve MPTP hydrochloride in 0.9% saline to a final concentration of 2 mg/ml. Prepare sufficient MPTP for the whole experiment to minimize exposure.
3. Fill 1-ml syringes attached to 21-G needles with ~0.5 ml of 2 mg/ml MPTP solution. Cap the needles, wrap syringes in Parafilm, and store in a transparent plastic container labeled "MPTP-Neurotoxin" with the user's name, contact details, and date. Store up to 2 months at −80°C until needed.
4. On day 1, 1 hr prior to injection, remove an appropriate number of syringes (1 syringe/animal) from the freezer and thaw at room temperature in a fume hood.
5. Weigh animals, accurate to 5 g.
6. Immediately prior to each injection, remove Parafilm wrap from syringe. Eject excess MPTP in syringe so the remainder will allow injection of 1 ml/kg, i.e., 2 mg/kg.
7. Inject marmoset with MPTP s.c. and return it to its cage.
8. Repeat steps 4 to 7 for an additional 4 days, so that each animal receives a total of five injections (one injection/day).

Following administration of MPTP, marmosets will begin to develop a parkinsonian-like condition. This will become progressively more marked over the first 4 to 6 weeks. During this time, some animals may require significant care and attention, including hand-feeding. After 6 weeks, functional improvement is usually noted and animals can generally feed and care for themselves unaided. By 14 weeks post-injection of MPTP, a stable parkinsonian state is achieved.

ASSESSMENT OF MARMOSETS RENDERED PARKINSONIAN BY ADMINISTRATION OF MPTP

BASIC PROTOCOL 3

Basic Protocol 2 can be used to produce animals for subsequent behavioral pharmacological studies to assess the anti-parkinsonian actions of therapies. In this instance, animals can be used in both acute or chronic studies using approaches to assess both parametric and non-parametric aspects of behavior, as described below. Activity counts are considered parametric as they display a Gaussian distribution, whereas the use of rating scales to assess the more complex behaviors produces data lacking a Gaussian distribution, therefore, data obtained with the use of such rating scales is considered non-parametric data.

Materials

Adult common marmosets (*Callithrix jaccus*), 300 to 400 g (Harlan)

Marmoset food (sliced banana, apples, and grapes)

Wire observation cages (0.8 × 0.8 × 0.7–m) with a Perspex sliding front (Harkes Industries) with a single branch situated at a diagonal slant across the center of the cage from the bottom left-hand corner at the cage front to the top right-hand corner at the rear of the cage

Video camera, video cassette, and tripod, or DVD recorder and DVDs
Infrared activity monitor and computer with appropriate data-logging software, optional

Additional reagents and equipment for MPTP treatment (see Basic Protocol 2)

1. Remove marmosets from their home cages and place them individually in wire observation cages for 4 hr/day on a daily basis for 4 weeks prior to MPTP treatment. Place a small dish of food (sliced banana, apples, and grapes) and a water bottle in each cage. Separate adjacent observation cages by curtains to prevent animals from interacting during trial periods. For each observation cage, align a video camera such that the cage front occupies the full frame. Attach each video camera to a DVD recorder in the same room such that the images received by the video camera are captured directly onto the DVD.

2. Following acclimation, assess motor activity on a daily basis until a stable baseline is recorded for 3 consecutive days.

   It typically takes 2 to 3 weeks of habituation to achieve 3 consecutive days of stable activity. Stable activity is defined as that which is not statistically significantly different from the previous 2 days activity.

   Assessment of motor activity can be done manually with the aid of a simple grid placed over the monitor playing the recording of the movement of the animals. Each time the animal moves accross a line of the grid, a count is noted. The authors use computer-based passive infrared activity monitors. These include an infrared-sensitive prism that detects movement of the warm-blooded animal as it moves around the cage. Activity counts are conveyed to a computer program as total counts per minute and can then be tabulated using a simple spreadsheet.

3. Treat animals with MPTP as described in Basic Protocol 2, steps 2 to 8.

4. Assess parkinsonian disability in 10-min observation periods every 30 min throughout the duration of the experiment using the rating scale detailed in step 5 (Fox et al., 2001, 2002; Henry et al., 2001; Savola et al., 2003).

   The duration of a typical experiment is 4 hr, but can be altered accordingly to allow for the differing duration of action of various drugs.

5. Determine the "Range of Movement" score (defined as the level of hypokinesia exhibited by the animals; with a higher score representing more severe symptoms of the disorder). Assign the maximum score achieved over the 10-min time period.

   0 = Running, jumping, climbing between cage walls, perch, and roof. The animal uses its limbs through a wide range of motion and activity.
   1 = Climbing up and down the walls of the cage or along perch.
   2 = Climbing onto perch or wall of cage.
   3 = Hopping on floor of cage.
   4 = Walking around floor of cage or eating from hopper on floor.
   5 = On perch or wall of cage, movement of limbs but no locomotion.
   6 = On perch or wall of cage, movement of head or trunk.
   7 = On the floor of the cage, movement of limbs but no locomotion.
   8 = On the floor of the cage, movement of head.
   9 = No movement.

6. Determine "Bradykinesia" score using the following scale. Assign the score representative of the level of behavior over the 10-min time period.

   0 = Normal initiation and speed of movement.
   1 = Slight slowing of movement.

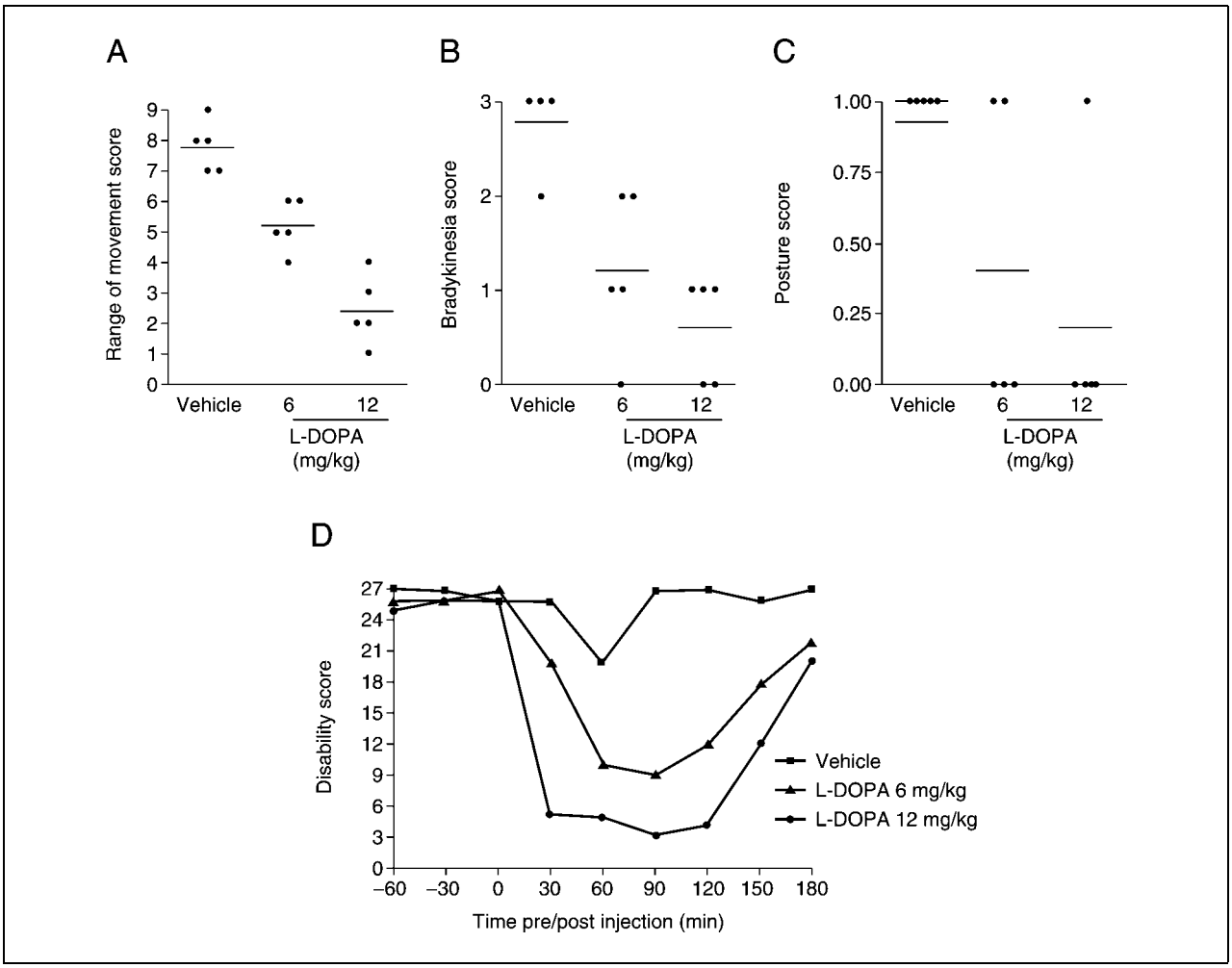

Figure 5.42.2 L-DOPA reverses parkinsonism in the MPTP marmoset. The effects of L-DOPA on (**A**) range of movement, (**B**) bradykinesia, and (**C**) posture in the MPTP-lesioned marmoset. Data are presented as individual values (points) with median (horizontal line) for a 10-min observation period 90 min after injection of vehicle or L-DOPA (6 to 12 mg/kg) (**A-C**). (**D**) Median disability scores per 10-min time period 60 min prior to and 180 min after injection of either vehicle or L-DOPA (6 to 12 mg/kg).

2 = Moderate slowing of movement, marked freezing, difficulty initiating, and maintaining movement.
3 = Prolonged freezing, akinetic, inability to move.

7. Determine "Posture" score.

   0 = Normal balance, upright posture, head held up.
   1 = Impaired balance, crouched posture, head down.

8. Determine "Disability" score using the following formula:

   Disability score = [(bradykinesia × 3) + (posture × 9) + (range of movement × 1)]

This score weights each parameter according to its impact on the overall well-being of the animal and provides a global parkinsonian disability rating.

Figure 5.42.2 illustrates data describing the anticipated level of parkinsonism resultant from MPTP administered in the manner described in Basic Protocol 2. Additionally, the anticipated effects of L-DOPA, an agent with anti-parkinsonian properties, are described. As these data are non-parametric, they should be presented appropriately (i.e., as median and range) and analyzed for statistical significance using non-parametric analyses.

ALTERNATE PROTOCOL

INDUCTION OF LEVODOPA-INDUCED DYSKINESIA IN THE MARMOSET MODEL OF PARKINSON'S DISEASE

One of the most common uses of the MPTP marmoset model (see Basic Protocol 2) is to produce animals exhibiting L-DOPA-induced dyskinesias of a similar nature to those displayed by humans following chronic exposure to the drug. Once rendered dyskinetic (see Support Protocol), these animals have been shown to have predictive power for the effects of potential anti-dyskinetic therapies in humans. Dyskinesia can be reliably produced in animals if levodopa therapy is administered immediately after parkinsonism is stabilized (8 to 12 weeks after administration of MPTP). Animals should be rendered parkinsonian as described in Basic Protocol 2 prior to induction of levodopa-induced dyskinesia. To render the animals dyskinetic, they receive bi-daily oral administration of an L-DOPA/benserazide solution (12.5/3.125 mg/ml, respectively), typically diluted in Gatorade. After 21 consecutive days of twice-daily levodopa therapy, animals typically exhibit dyskinesia that is stable with time and reproducible on each challenge with levodopa, provided levodopa therapy is maintained with a frequency of at least three times a week.

SUPPORT PROTOCOL

ASSESSMENT OF DYSKINESIA PSYCHOMIMETIC BEHAVIORS FOR BEHAVIORAL PHARMACOLOGY EXPERIMENTS

At the present time, there is no single standardized scale for quantifying dyskinesia in non-human primates (see Petzinger et al., 2001, for a review of some of the rating scales used). Dyskinesia in marmosets has typically been evaluated according to the criteria detailed below. See Pearce et al. (1995) for the original description of levodopa-induced dyskinesia (LID) in marmosets.

The presence of chorea (rapid, random flicking movements) and dystonia (abnormal, sustained posturing) is scored using the following, specifically designed, dyskinesia scoring system:

0 = Absent
1 = Mild: fleeting and rare dyskinetic postures and movements
2 = Moderate: more prominent abnormal movements, without impinging significantly on normal behavior
3 = Marked: frequent, sometimes continuous, dyskinesias that impinge on normal activity
4 = Severe: virtually continuous dyskinesias that are disabling and that replace normal activity

In the marmoset, it can sometimes be difficult to distinguish dyskinesia that is unequivocally dystonic rather than choreic. Thus, it is usually sufficient to consider dyskinesia as an entity in this species. However, larger primates, such as macaques, will exhibit dyskinesia that is more easily distinguishable as choreic versus dystonic, than is the case for marmosets, thus it is possible to make this distinction with more confidence in these larger species.

The materials and experimental protocol are as described in Basic Protocol 3 for the assessment of parkinsonian symptoms. Dyskinesia is usually assessed in 10-min observation periods every 30 min throughout the duration of the experiment. The final score for dyskinetic behaviors in each 10-min observation period represents the predominant level of behavior over the whole 10-min period.

Figure 5.42.3 illustrates the anticipated dyskinesia score, and the expected effect of an antidyskinetic agent (amantadine), following administration of L-DOPA to marmosets rendered parkinsonian and dyskinetic as described.

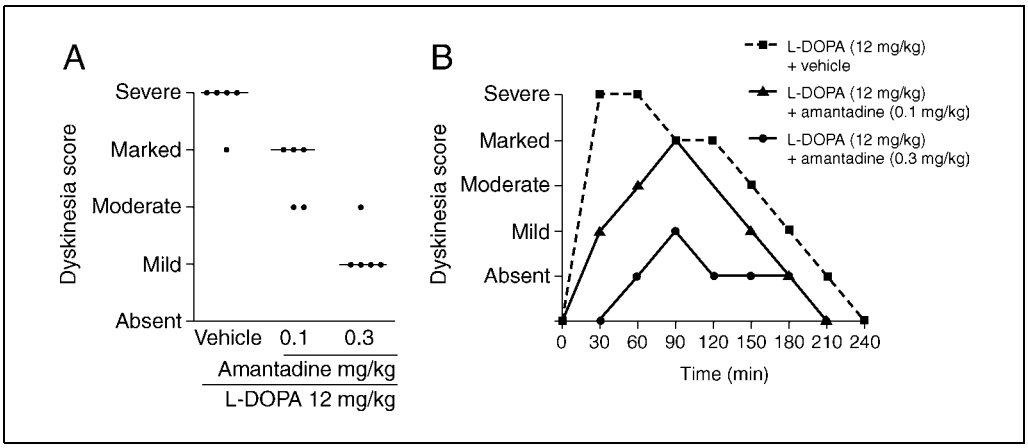

Figure 5.42.3 Amantadine reverses dyskinesia induced by L-DOPA in the MPTP-treated marmoset. Data are presented as (**A**) individual values (points) with median (horizontal line) during a 10-min observation period 60 min after injection of L-DOPA (12 mg/kg) + vehicle or L-DOPA + amantadine (0.1 to 0.3 mg/kg), and (**B**) median activity scores per 10-min time period from 0 to 240 min after injection of either L-DOPA + vehicle or L-DOPA + amantadine (0.1 or 0.3 mg/kg).

MPTP-lesioned marmosets treated chronically with levodopa also develop abnormal behaviors reminiscent of those observed following administration of a psychotomimetic (Fox et al., 2004). These may be correlated to psychosis and/or the dopamine disregulation syndrome observed in Parkinson's disease. These behaviors include:

a. Hyperkinesia: Fast, driven, relentless running movements with variable direction. Usually jumping, running from one side of cage to the other.
b. Response to apparent non-stimuli: Tracking—following apparent stimuli; staring—head still, looking in one direction for an extended period.
c. Excessive and repetitive scratching.
d. Stereotypies: Side-to-side jumping with quick, repeated jumping movements; quick, darting side-to-side head movements; purposeless running, jumping in circles—same movement repeated over and over; repetitive grasping at bars.
e. Vocalizing.

The presence or absence of each of these abnormal behaviors is assessed and rated 1 (present) or 0 (absent), every minute for 10 min, two times/hr, over a period of 4 hr. The scores are then summed to yield a total value.

COMMENTARY

Background Information

This unit describes two methods for selectively destroying dopaminergic neurons in mice and marmosets to create animal models of Parkinson's disease. The models are especially valuable for studying the changes in brain function that occur over time following an almost complete loss of dopamine and screening agents that have the potential to be neuroprotectants or that may restore lost dopamine function. In addition, in the case of the primate model, the potential for symptomatic treatment to alleviate parkinsonism without producing dyskinetic side effects can be assessed.

These models have also been employed to assess potential neuroprotective or neurorestorative treatments. However, a drawback to the models described herein is that they differ from the human condition by not displaying the progressive nature of the disorder. In recent years, MPTP models have been introduced that display a progressive loss of dopaminergic function (Bezard et al., 1997a,b). These newer models are better suited than those described in this unit for studying disease progression

and for screening test agents that may be disease-modifying agents. They are also more appropriate for assessing pre-symptomatic compensatory mechanisms and diagnostic agents.

Critical Parameters

Safety issues relating to use of MPTP

As MPTP is known to cause parkinsonism in humans, all precautions should be taken to minimize exposure to this agent when using these protocols (Przedborski et al., 2001). Approval for the use of this neurotoxin from the institutional Occupational Health and Safety Committees (or similar agency) should be obtained.

The safe handling of MPTP involves the following:

1. Rooms with negative pressure ventilation should be dedicated for preparation and administration of MPTP. Animals administered MPTP should be kept in such rooms for at least 5 days after the last injection of this agent (see below). Such rooms should have signage indicating their use, e.g., "Entry Restricted to Authorized Personnel."

2. Staff working with MPTP must be trained in the use of hazardous substances and must voluntarily provide their informed, written consent to participate in these studies.

3. Stock solutions of MPTP should be prepared in a fume hood.

4. If possible, animals should be injected with MPTP in a fumehood.

5. Protective equipment should be used by personnel involved in the preparation of MPTP solutions, the administration of MPTP, or any activity performed in the dedicated MPTP room during the period of MPTP administration. Such equipment should include a full-face respirator and disposable overalls with a hood and elasticized cuffs.

6. If possible, when preparing MPTP solutions, dissolve the entire contents of the bottle at once rather than attempting to weigh out portions, which increases the risk of spillage and aerosol generation.

7. If possible, prepare a sufficient amount of MPTP stock solution for the whole study at once and freeze until needed to minimize the risk of accidental exposure.

8. Any spillage or minor contamination with MPTP can be adequately controlled by thorough cleaning with a 1% bleach solution. It has been demonstrated that an equal volume of a 1% bleach solution is able to completely detoxify a 5 mg/ml MPTP solution within 5 min at room temperature (Przedborski et al., 1996). To decontaminate following a spill, spray the entire contaminated area with a 1% bleach solution and leave for 10 min. Absorb the spill with plastic-backed absorbent pads and dispose as hazardous waste.

9. Procedures for dealing with contamination or accidental self-injection/ingestion should be established and practiced prior to undertaking any experiments and must be clearly posted in the MPTP room.

10. Emergency rooms in local hospitals should be made aware of the use of MPTP so treatment with an MAO inhibitor such as selegiline can be quickly administered in case of ingestion or inhalation.

11. The MPTP room should be decommissioned at the end of the study, but no sooner than 5 days after the last MPTP administration. The major risk of contamination comes from contact with the animal, cage, urine, and feces following administration of the toxin. Although MPTP is rapidly metabolized, 5 days is recommended as a conservative estimate of the period of greatest risk. Upon decommissioning, all hazardous waste in the room must be incinerated and all equipment and surfaces must be cleaned with a 1% bleach solution.

Troubleshooting

Mouse protocol

If the lesions obtained show unacceptable variability, it is possible that the strain of mouse used is insensitive to MPTP. C57BL/6 mice are reliably sensitive to MPTP and can be used to verify that the protocol is being performed correctly. In addition, male mice should be used since they are more sensitive than females to MPTP (Miller et al., 1998).

If mortality is high (>25%) it may be that the animals have not had sufficient time to acclimate to their surroundings or that they are outside the recommended weight range for the protocol. They should be allowed at least 7 days acclimation before experimentation and should weigh 25 to 30 g (8 weeks old).

Marmoset protocol

In the primate protocol described, the major factor influencing the ability to produce a stable parkinsonian state appears to be body weight. Animals weighing >400 g are more likely to develop a very severe parkinsonian syndrome, which can make maintenance of

Table 5.42.1 Anticipated Actions of Key Reference Compounds in the MPTP-Lesioned Marmoset Model of PD and LID

Compound	Chemical name	Mechanism of action	Dose (mg/kg)	Anti-parkinsonian	Anti-dyskinetic
Amantadine	Tricyclo[3.3.1.13.7]dec-1-ylamine hydrochloride	Glutamate antagonist	0.3[a]	Yes	Yes
Apomorphine	(R)-5,6,6a,7-Tetrahydro-6-methyl-4H-dibenzo[de,g]quinoline-10,11-diol hydrochloride	Dopamine receptor agonist	28[b]	Yes	No
Bromocryptine	2-Bromo-α-ergocryptine methanesulfonate	Dopamine D2 receptor agonist	1.0[c]	Yes	No
Fipamezole	4-(2-Ethyl-5-fluoro-2,3-dihydro-1H-inden-2-yl)-1H-imidazole	α2 adrenergic receptor antagonist	10[d]	No	Yes
L-DOPA	L-3,4-Dihydroxyphenylalanine	Dopamine replacement	20[e]	Yes	No
Levetiracetam	(S)-alpha-ethyl-2-oxo-1-pyrrolidineacetamide	SV2a modulator	60[a]	Yes	Yes
Selegiline	(R)-(-)-*N*-a-dimethyl-*N*-2-propynylbenzeneethanamine hydrochloride	MAO-B inhibitor	10[f]	Yes	No

[a]Hill et al., 2004.
[b]Blanchet et al., 1997.
[c]Pearce et al., 1998.
[d]Savola et al., 2003.
[e]Jenner et al., 1984.
[f]Kupsch et al., 2001.

appropriate levels of food and fluid intake difficult. On the other hand, animals weighing <300 g show lower sensitivity to MPTP and can recover to near normal levels of behavior only a few months after MPTP administration. In marmosets of this weight range (300 to 400 g), gender does not appear to have a significant effect on the ability of MPTP to induce parkinsonism.

Anticipated Results

Listed in Table 5.42.1 are results obtained using both anti-parkinsonian and antidyskinetic agents in the MPTP-lesioned marmoset.

Time Considerations

Mouse protocol

It usually requires 1 working day to treat the animals with MPTP (20 mg/kg s.c., four times/day). Therefore, if the experimental day commences at 9 am (when prepared MPTP syringes are removed from the freezer to thaw, and all animals are weighed), injections would occur at 10 am, 12 noon, 2 pm, and 4 pm. The amount of time required over subsequent days is dependent on the dosing regime of the agents to be tested.

Marmoset protocol

If the animals are to be used for behavioral observations, the initial time constraint of this procedure is the ~4-week acclimatization period needed prior to administration of MPTP. This comprises 2 weeks of acclimatization to the new housing cages following arrival, and the ~2 weeks habituation to the observation cages required to achieve 3 consecutive days stable activity as discussed in Basic Protocol 2. The development of parkinsonism in MPTP-treated marmosets as described in Basic Protocol 2 occurs over a period of ~3 to 4 months. During the early stages of this period, the animals typically require considerable attention to ensure that they do not suffer excessive weight loss due to an impaired ability to eat. Thus, Basic Protocol 2 can be very labor-intensive for 3 to 4 months. If the animals are to be rendered dyskinetic, L-DOPA treatment to induce dyskinesia should commence only after a stable parkinsonian state is established based on the results of repetitive behavioral testing. The period of chronic L-DOPA treatment required to produce stable, reproducible dyskinesias is typically 8 to 12 weeks. Thus, these experiments are not only time-intensive, but can also be relatively labor-intensive.

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