Dec 14, 2023

Standard Operating Procedure (SOP) for Combined ICA and Systemic Administration of MPTP: The Overlesioned (Bilateral Asymmetric) Non-human Primate Model

DOI
dx.doi.org/10.17504/protocols.io.dm6gp3zw5vzp/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.dm6gp3zw5vzp/v1

Protocol Citation: Robert S Turner 2023. Standard Operating Procedure (SOP) for Combined ICA and Systemic Administration of MPTP: The Overlesioned (Bilateral Asymmetric) Non-human Primate Model. **protocols.io https://dx.doi.org/10.17504/protocols.io.dm6gp3zw5vzp/v1**

Protocol status: Working
We use this protocol and it's working

Created: August 29, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 87061

Keywords: ASAPCRN, Non-human primate, MPTP, MPTP-lesion, stable parkinsonian syndrome, occurring parkinson, damage to the mitochondrial respiratory system, systemic administration of mptp, dopamine, administration of mptp, basis of the toxic effect, mptp administration, resultant abnormalities in brain physiology, mitochondrial respiratory system, oxidase, brain physiology, axonal transport, tetrahydropyridine, mptp, toxic effect, mao, mitochondria

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-020519

Abstract

This standard operating procedure (SOP) describes safe methods for the use of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in non-human primates (adult macaque monkeys).

In both humans and nonhuman primates, the administration of MPTP produces a stable Parkinsonian syndrome. Upon entering the brain, MPTP is converted into the toxic metabolite MPP^+ by the enzyme monoamine oxidase B (MAO-B). MPP^+ accumulates selectively in neurons possessing a dopamine (DA) uptake system and is further concentrated in mitochondria. The basis of the toxic effects of MPP^+ is only partially understood; retrograde axonal transport, melanin binding, and damage to the mitochondrial respiratory system have been implicated in the pathogenesis of injury to DA neurons. MPTP administration reproduces in monkeys a syndrome that closely approximates naturally occurring Parkinson's disease (PD) with respect to the pattern of DA cell loss, the resultant abnormalities in brain physiology, and ultimately, clinical symptoms.

This SOP describes the procedures for the combined internal carotid artery (ICA) and systemic (intravenous or intramuscular) administration of MPTP in non-human primates. This model produces an overlesioned or bilateral asymmetric model, induced by unilateral ICA plus systemic administration.

This protocol has been adapted from Bankiewicz et al. (1999).

Guidelines

Methods for the safe conduct of experimental procedures

- ***Training of Personnel:*** Only laboratory personnel who are trained in handling hazardous agents and in MPTP safety procedures are allowed to prepare and administer MPTP or to monitor the animals during the high-risk period (3 days post-MPTP injection). Any personnel involved in these procedures needs to be fully informed of the risks associated with exposure to MPTP, provided with an opportunity to consent, and not be coerced into taking on MPTP-associated duties. To participate in this work, personnel are required to read two standard references on the safe use of MPTP: "Recommended safe practices for using the neurotoxin MPTP in animal experiments" Lab Anim Sci 38:563-567, 1988; and "The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP): a technical review of its utility and safety" J Neurochem 76:1265-1274, 2001. Personnel are not allowed to perform MPTP-related tasks by themselves until the PI has provided individual training and then observed the individual perform the required safety procedures correctly on at least two separate occasions.
  At the University of Pittsburgh, lab personnel who plan to work with MPTP must also read the University of Pittsburgh Employee Health "MPTP Information Form" and submit an "MPTP Occupational Consent" and Selegiline-related forms.

- ***Personal Protection Equipment:*** PPE is worn during all procedures involving MPTP, MPTP-exposed animals, or MPTP-contaminated equipment. This includes solution preparation, surgery, and the 3 days post-MPTP injection. The PPE will consist of a one-piece garment (with an attached hood), elasticized wrists, and attached boots made of chemically- and biologically inert, non-absorbent material (Tyvek fabric). Personnel wear a full- or half-mask respirator with a removable HEPA filter (NIOSH/MSHA-approved) which has been fit-tested to the individual. Personnel using respirators undergo annual health assessments and a fit test for respirator use. Personnel also wear splash-proof goggles or a face shield and puncture-resistant gloves during all procedures.

- ***MPTP Storage, Handling, and Solution Preparation:*** MPTP is purchased from a commercial source (Sigma-Aldrich, St. Louis, MO) in quantities of **10 mg** in glass septum bottles within an outer vacuum-sealed container. These containers are stored at room temperature in a locked cabinet labeled: **'DANGER: MPTP – Neurotoxin'**. Solutions are mixed in a chemical fume hood. Prior to opening a container, lab personnel perform a safety check to confirm that the fume hood is operating correctly, the work surface within the fume hood is lined with plastic-backed absorbent pads and personnel wears full PPE. A spray bottle filled with 1% bleach solution is available within the fume hood to be used to degrade MPTP in case of a spill. The method described for the preparation of the MPTP solution reduces the potential for exposure to free MPTP powder because MPTP is removed from a septum vial only after it is dissolved in sterile saline. After the solution is prepared and loaded into the syringe, the materials and PPE used during solution preparation are sprayed with 1% bleach and disposed of as biohazardous waste. Prior to discarding the used MPTP vial as biohazardous liquid waste, bleach is injected into the vial.

- ***Animals should be adults and motor function should be assessed before MPTP administration.***

- ***MPTP Dose Limitations:*** Institutional Animal Care and Use Committees (IACUC) or veterinary SOPs may limit the number of MPTP administrations and/or the maximal dose of MPTP that can be administered to animals.

- ***Administration of MPTP Solution:*** For ICA and IV or IM administration, a sign is posted on the operating or procedure room door warning **'DANGER: MPTP – Neurotoxin Use Area – Entry Restricted.'** (This sign must also list emergency contact information for two lab personnel.) Full PPE is worn during the injection procedures. In the case of leakage of injectate from an IV injection site, the skin around the injection site will be wiped with a gauze pad dampened with 1% bleach solution. MPTP-contaminated syringes are filled with 1% bleach solution and then discarded as biohazardous waste along with surgical drapes, absorbent pads, and PPE.

- ***Animal Care Post-MPTP Administration:*** For at least **3 days** following the administration of MPTP, animals are housed in an isolation room that is physically separated from other animals). During this quarantine period, only trained laboratory personnel (and *not* the general animal care staff) provide care to the animal. Lab personnel supply the animal with standard food and water rations plus enhanced enrichment throughout the period of isolation. The base of the cage, the drop pan, and the floor surrounding the cage are lined with plastic-backed absorbent pads. Posted on the door of the quarantine space there is a sign warning: '**DANGER: MPTP – Neurotoxin Use Area – Entry Restricted.'** PPE clothing will be worn by laboratory personnel who enter the enclosure to feed or otherwise interact with the animal. Every day for three days, laboratory personnel wearing full PPE dampen the contaminated bedding material and absorbent pads with 1% bleach solution, allow the solution to soak for 10 minutes, and then place the materials in a plastic bag. After sealing this bag, it is wiped with 1% bleach and then placed in a second biohazard bag before disposal as biohazardous waste. At the end of the confinement period, the animal can be returned to its home cage. The cages are sprayed liberally with 1% bleach solution and allowed to soak for 10 minutes before being washed with detergent and rinsed thoroughly.

- ***Preparation for Accidental Exposure:*** MAO-B is the enzyme that converts MPTP to its toxic metabolite MPP+. Therefore, MAO-B inhibitors prevent the conversion of MPTP, thereby preventing neurotoxicity. Deprenyl (selegiline), an MAO-B inhibitor that has been shown to protect animals from MPTP toxicity, will be kept in the first aid kit in the investigator's lab. In case of accidental exposure of lab personnel to MPTP, a large dose of deprenyl will be administered immediately (four 5 mg tablets). Ongoing treatment with deprenyl (5 mg twice a day) will be suggested in consultation with the individual's personal physician.

References:

Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, Akram M (2001). "The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP): a technical review of its utility and safety". *Journal of Neurochemistry 76*, 1265-1274.

Yang SC, Markey SP, Bankiewicz KS, London WR, Lunn G (1988), "Recommended safe practices for using the neurotoxin MPTP in animal experiments." *Laboratory Animal Science 38*, 563-567.

Materials

Personal Protective Equipemnt

- Disposable one-piece garment (with an attached hood), elasticized wrists, and attached boots made of chemically- and biologically inert, non-absorbent material (Tyvek fabric)
- Two pairs of chemical-resistant (nitrile) gloves that cover the wrist of the coverall
- Puncture resistant gloves
- Half- or full-mask respirator with a removable HEPA filter (NIOSH/MSHA-approved) which has been fit-tested to the individual. Personnel using respirators undergo annual health assessments and a fit test for respirator use
- Splash-proof goggles or a face shield (if using a half-mask respirator)

Materials

- Sterile saline: 0.9% (w/v) NaCl
- 10 mg vial of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine HCl (MPTP·HCl; Sigma)
- Adult macaques (Rhesus or Cynomolgus)
- Ketamine/xylazine
- Isoflurane
- Betadine
- 70% (v/v) ethanol
- 1% bleach solution in water
- Peroxide
- 10- and 60-ml sterile syringes
- 10- or 30-ml sterile vials
- Scale
- Intravenous (i.v.) extension set
- Electric shaver
- Alcohol pads
- 22-G i.v. catheter
- Tracheal tube
- Surgical table
- Absorbent blue bench pads
- Stretch gauze and cotton swabs
- Sterile surgical tools: scalpel, large forceps, delicate curved forceps, scissors, needle holders, retractors, mosquito hemostats
- 27-G sterile needle
- Infusion pump (fitted for a 60-ml syringe)
- 3-0 Vicryl with needle
- Plastic, transparent millimeter-scale ruler
- 22-G angiocatheter
- Heated water pad

- Drapes
- 2/0 silk suture
- Towel clamps
- Biohazard disposal bags and boxes
- Selegiline (5 mg tablets, optional)

Troubleshooting

Safety warnings

The most hazardous operations in conducting MPTP animal experiments are the preparation, handling, and injection of concentrated solutions of MPTP. The concentration of MPTP in the solution used for ICA administration is very low (0.05 to 0.07 mg/ml) and therefore is relatively safe. However, the solutions for IV or IM administration (even in small volumes) are much more concentrated, and additional caution will be taken during its handling. Management of these risks requires that solutions be prepared in a ventilated fume hood with precautions to catch and dispose of any spilled solution and that personnel wear appropriate personal protective equipment at all times.

The second primary hazard from these experiments is the potential for exposure of personnel to MPTP or its metabolites that have been excreted by an animal in the days following MPTP administration. Management of this risk requires an understanding of how MPTP is metabolized and excreted following its administration. It is worth emphasizing here that MPTP is the excreta of highest concern because only MPTP can cross the blood-brain barrier and thus endanger personnel who are exposed to it. The metabolite MPP+ is highly toxic to dopamine neurons when it is injected directly into the brain, but when injected peripherally, only very high doses of MPP+ (e.g., 25 mg/kg i.p.) produce detectable toxicity and then only peripherally. Studies have shown that following MPTP administration to a monkey, only the interior surfaces of the animal's cage and surfaces that the animal and/or its excreta can physically touch (e.g., bedding, food hoppers, drinking bottles) are contaminated with MPTP and its metabolites. At two days post-injection, 70% of the total injected dose of MPTP and its metabolites can be recovered from inside the cage, from urine, and from feces. Of this, only 2% consists of MPTP itself, while the rest consists of metabolites such as MPP+. Unmetabolized MPTP is excreted primarily in the first day post-administration, while metabolites are excreted up to 3 days post-injection. There is no evidence that MPTP or its metabolites are still being excreted after 3 days post-MPTP administration. MPTP is excreted primarily in the urine in an ionized (i.e., non-volatile) form. Thus, excreted MPTP is well absorbed by animal bedding material and/or absorbent pads. Less than 0.01% of the total injected dose of MPTP can be detected as volatile MPTP. In summary, the potential risks of exposure to MPTP following its administration are through direct contact with the animal, the inner surfaces of the animal's cage, and its soiled bedding material. There is minimal risk from exposure due to airborne forms of MPTP. The period of maximal risk of MPTP contamination is from the moment of injection to 3 days post-MPTP injection.

Ethics statement

Experiments involving animals must always be conducted according to internationally accepted standards and must have approval from an Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s). Approval must be obtained *before* engaging in any animal experiments, such as those described in this protocol. The protocol described here has been approved by the University of Pittsburgh Animal Care and Use Committee (IACUC) and is subject to review and refinement every 3 years.

Before start

- ***Confirm IACUC approval and training for all personnel involved in the procedures.***
- ***Confirm availability for post-MPTP isolation housing and care:*** The attending veterinarian and the responsible animal care supervisor must be notified at least **3 days** prior to each instance of MPTP use (i.e., prior to each ICA surgery and prior to any course of IV or IM administration). Before proceeding, ensure that appropriate isolation housing is available for animals post-MPTP administration.
- ***Confirm the availability of required PPE, materials, and selegiline for administration in case of accidental personnel exposure.***

Receipt and storage of MPTP

1 Place the package with MPTP into a chemical fume hood.

2 Don appropriate personal protective equipment (Tyvek suit, half-mask respirator, bonnet, face shield, 2 pairs of nitrile gloves) and inspect the package to ensure no damage.

2.1 If all packaging and bottles are intact, remove the MPTP bottle from the packaging and safely store it in a locked cabinet.

2.2 If an MPTP bottle is damaged, add 1% bleach solution to the vial to inactivate the MPTP. Discard the MPTP in a dedicated biohazard container and contact the **Environmental Health and Safety Office** to dispose of the biohazard container.

MPTP dosing for ICA administration

3 MPTP is toxic during only the first pass through the brain. This is because MPTP also undergoes conversion to MPP+ in the periphery, after which it cannot cross the blood-brain barrier. Thus,
for administration, **the dose of MPTP is calculated according to brain size**, which tends to be constant over a wide range of body weight, instead of per body weight.

3.1 Small animals **(3 - 5 kg)**: 2.5 mg **to** 2.5 mg **MPTP**.

3.2 Medium-sized **(6 - 10 kg)** animals: 3 mg **to** 3.5 mg **MPTP.**

3.3 Large animals **(>10 kg):** 4 mg **MPTP**.

Preparation of MPTP solution for ICA administration

4 Prepare the MPTP solution by first infusing 1 mL sterile saline into a septum-sealed sterile vial containing 10 mg MPTP-HCl powder. After the powder is fully dissolved,

aspirate the 1 mL solution into a syringe containing 9 ml of sterile saline (**final concentration:** [M] 1 mg/mL **MPTP**).

5 Transfer the entire 10 mL MPTP solution to a septum-sealed sterile vial (10 ml or 30 ml).

6 For medium or large animals, fill a sterile 60-ml syringe with 3 mL to 4 mL of the MPTP solution (3 mg to 4 mg MPTP) and then 56 mL to 57 mL of sterile saline. The final concentration in the syringe will be 3 to 4 mg/60 ml or [M] 0.05 mg/mL to [M] 0.067 mg/mL MPTP.

7 Attach the filled 60 mL syringe to an i.v. extension set.

Preparation of the animal for ICA administration of MPTP

8 Anesthetize a macaque monkey with ketamine (**10 mg/kg**) and xylazine (**1 mg/kg**) in the home cage. Transfer the animal to a procedure room.

9 Shave the calves and neck region of the animal using an electric shaver. Palpate the calf muscle and identify the saphenous vein.

10 Clean the skin with an alcohol pad. Using a 22-G i.v. catheter, cannulate the vessel and flush with saline.

11 Intubate the animal with a 3.5 mm to 5 mm (inside diameter) tracheal tube, according to the size of the animal.

12 Transfer the animal to a surgery room, place it on a surgical table with absorbent blue bench pads, and a heated water pad, or Bair Hugger (3M) at 37 °C. Maintain on isoflurane anesthesia (e.g. 1.5% with an oxygen flow of ~0.3 liters/min).

13 Hyperextend the head on the surgical table by placing stretch gauze through the canines and applying gentle retraction. Secure the head in position by tying the gauze to the surgery table.

14 Cover the animal completely with a series of sterile drapes. Cut out an opening in the drape for the neck incision and secure drapes with towel clamps. Scrub the surgery site with Betadine followed by 70% ethanol.

15 Make a midline incision through the skin of the neck with a sterile scalpel.

16 Using blunt dissection technique, locate and open the carotid sheath, exposing the common carotid artery, internal jugular vein, and vagus nerve. Isolate the common carotid artery below the carotid bifurcation.

17 Locate the superior thyroid artery and the external carotid artery and temporarily clamp the vessels using mosquito hemostats.

18 Attach a 27-G needle to the i.v. extension tubing and drain all the air from the needle by activating an infusion pump for several seconds. Drain all excess MPTP onto a sponge soaked with 1% bleach solution.

Intracarotid artery (ICA) infusion

19 Insert the 27-G needle (with attached extension set and 60-ml MPTP syringe) into the internal carotid artery in a direction retrograde to the direction of blood flow.

20 Infuse the entire volume from the syringe at 4 ml/min (3 mg to 4 mg MPTP).

21 Remove the vascular clamps (or mosquito hemostat) from the superior thyroid and external carotid arteries. Withdraw the needle from the common carotid artery and apply pressure for 00:05:00 (until bleeding has stopped). 5m

22 Clean the wound with peroxide, close the incision site at the neck with 3-0 Vicryl, and clean the sutured area.

23 Allow the animal to recover from anesthesia (~15 minutes) before placing in a cage in the isolation room for 72:00:00 post-MPTP injection. 3d

Intravenous (IV) and Intramuscular (IM) Administration of MPTP

24 Systemic administration (IV or IM) will be used when it is necessary to supplement the effects of ICA MPTP administration so as to produce a chronic moderate Parkinsonian syndrome.

25 Following ICA MPTP infusion, house animals in their home cage for at least 1 week and assess motor function before further IV or IM administration of MPTP.

Intravenous (IV) Administration of MPTP

26 Weigh the animal to determine the total dose of MPTP required. For IV administration the recommended dose is **0.3 mg/kg** body weight.

27 Anesthetize the animal with ketamine (**10 mg/kg**) and xylazine (**1 mg/kg**) in its home cage. Transfer the animal to a procedure room.

28 Shave the calves using an electric shaver. Palpate the calf muscle and identify the saphenous vein. Clean the area with an alcohol pad.

29 Using a 22-G angiocatheter, cannulate the vessel and flush with sterile saline.

30 Fill a sterile 6 mL syringe with the appropriate volume of [M] 1 mg/mL MPTP solution (**0.3 ml/kg** or **0.3 mg/kg**) and infuse the MPTP into the vein over 00:03:00 . 3m

31 Flush the line with 3 mL sterile saline.

32 Remove the needle and apply pressure to the saphenous vein until bleeding stops.

33 The animal will be allowed to recover from anesthesia (~15 minutes) and then be placed in a cage in an isolation room for 3 days (72:00:00) post-MPTP injection 3d

Intramuscular (IM) Administration of MPTP

3d

34 Move the animal to a cage in an isolation room prior to IM MPTP administration

35 Lightly anesthetize the animal with ketamine (**4 - 10 mg/kg**) and transfer to a procedure table in the isolation room or to a procedure room.

36 Weigh the animal to determine the total dose of MPTP required (**0.3 - 1 mg/kg**)

37 Inject the MPTP into the thigh muscle at one or multiple sites (if the volume is larger than 1 ml) using sterile syringes.

38 Allow the animal to recover from light sedation (5 - 10 minutes) and place in a cage in an isolation room for 3 days (72:00:00) post-MPTP injection

3d

Animal Care Post-MPTP

3d

39 For at least 72:00:00 following the administration of MPTP, house the animal in an isolation room that is physically separated from other animals in the colony that are not receiving MPTP).

3d

40 Given safety concerns (i.e. onset of motor signs), animals should not be housed in double-tier cages during MPTP administration and isolation period.

41 Post signs warning: '**DANGER: MPTP – Neurotoxin Use Area – Entry Restricted'** on the doors to the quarantine space.

42 During this quarantine period, only trained laboratory personnel (and *not* the general animal care staff) can provide care to the animal. Lab personnel supply the animal with standard food and water rations plus enhanced enrichment throughout the period of isolation.

43 Laboratory personnel who enter the enclosure to feed or otherwise interact with the animal need to wear appropriate PPE. The PPE consists of a one-piece garment (with an attached hood), elasticized wrists, and attached boots made of chemically- and biologically inert, non-absorbent material (Tyvek fabric). Personnel also need to wear a full- or half-mask respirator with a removable HEPA filter (NIOSH/MSHA-approved) which has been fit-tested to the individual. Personnel using respirators undergo annual

health assessments and a fit test for respirator use. Personnel also wear splash-proof goggles or a face shield and puncture-resistant gloves during all procedures.

44 Line the base of the cage, the drop pan, and the floor surrounding the cage with plastic-backed absorbent pads.

45 Every day for three days, laboratory personnel wearing full PPE dampen the contaminated bedding material and absorbent pads with 1% bleach solution, allow the solution to soak for 10 minutes, and then place the materials in a plastic bag. After sealing this bag, it is wiped with 1% bleach and then placed in a second biohazard bag before disposal as biohazardous waste and incinerated.

46 At the end of the confinement period, returned to its home cage. Spray the cages liberally with 1% bleach solution and allow to soak for 10 minutes. Once the animal(s) have been transferred to clean cages, and the room has been decontaminated, the use of special MPTP PPE is not required, and the room may be cleaned according to standard husbandry procedures.

Protocol references

Bankiewicz KS, Sanchez-Pernaute R, Oiwa Y, Kohutnicka M, Cummins A, Eberling J (1999). "Preclinical models of Parkinson's disease." *Current Protocols in Neuroscience 9 (1)*, 1.5.1-9.4.32.
Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, Akram M (2001). "The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP): a technical review of its utility and safety". *Journal of Neurochemistry 76*, 1265-1274.
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