Feb 20, 2025 Version 1

Stereotaxic Surgery for Viral Injection and Mild 6-OHDA Lesion Model in Mice V.1

DOI
dx.doi.org/10.17504/protocols.io.q26g7m2n1gwz/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.q26g7m2n1gwz/v1

Protocol Citation: Marta Graziano, Ioannis Mantas, Konstantinos Meletis 2025. Stereotaxic Surgery for Viral Injection and Mild 6-OHDA Lesion Model in Mice. **protocols.io https://dx.doi.org/10.17504/protocols.io.q26g7m2n1gwz/v1**

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

Created: February 20, 2025

Last Modified: March 26, 2025

Protocol Integer ID: 123079

Keywords: stereotaxic surgery for viral injection, ohda lesion model in mice, performing stereotaxic surgery, stereotaxic surgery, viral injection, ohda lesion model, drug injection, postoperative care, surgery

Abstract

This protocol outlines the procedure for performing stereotaxic surgery for viral or drug injections and the establishment of a mild 6-OHDA lesion model in mice. The protocol ensures accurate targeting and minimal discomfort, while also detailing postoperative care and monitoring for effective recovery.

Materials

Anesthetics and Analgesics:
- Isoflurane (4% induction, 1.5-2.5% maintenance)
- Buprenorphine (0.1 mg/kg, subcutaneous)
- Xylocaine/Lidocaine (4 mg/kg, local)
- Carprofen (5 mg/kg, subcutaneous)

Pharmacological Agents for 6-OHDA Lesion:
- Desipramine (25 mg/kg, i.p.; Cat# D3900, Sigma–Aldrich)
- Pargyline (5 mg/kg, i.p.; Cat# P8013, Sigma–Aldrich)
- 6-OHDA (0.6 µg/µL in 0.02% ascorbate; Cat# 162957, Sigma–Aldrich)

Surgical Equipment:
- Stereotaxic frame (Harvard Apparatus, Holliston, MA)
- Quintessential Stereotaxic Injector (Stoelting, Wood Dale, IL)
- Capillary needle for viral injection and 6-OHDA injection
- Heating pad
- Eye ointment
- Surgical scissors and forceps
- Sterile gauze and cotton swabs

Sterile Supplies:
- Surgical gloves
- Face mask
- Sterile drapes

Troubleshooting

Animal Preparation

1 Anesthesia and Analgesia:

1.1 Induce anesthesia with 4% isoflurane in an induction chamber.

1.2 Maintain anesthesia at 1.5-2.5% isoflurane throughout the procedure.

1.3 Administer Buprenorphine (0.1 mg/kg, subcutaneous) for preoperative analgesia.

1.4 Apply Xylocaine/Lidocaine (4 mg/kg) locally to the surgical site.

2 Pre-treatment for 6-OHDA Lesion Model:

2.1 Administer Desipramine (25 mg/kg, i.p.) to protect noradrenergic neurons.

2.2 Administer Pargyline (5 mg/kg, i.p.) to inhibit monoamine oxidase and prolong 6-OHDA action.

2.3 Wait for at least 30 minutes after pretreatment before proceeding with surgery.

3 Positioning and Stabilization:

3.1 Place the anesthetized mouse in the stereotaxic frame.

3.2 Secure the head using ear bars to ensure stability.

3.3 Maintain body temperature using a heating pad set to approximately 37°C.

3.4 Apply eye ointment to protect the eyes from drying.

Surgical Procedure

4 Incision and Exposure:

4.1 Make a midline scalp incision to expose the skull.

4.2 Clean the exposed area using sterile saline or ethanol.

5 Coordinate Identification and Drilling:

5.1 Identify the target injection site using stereotaxic coordinates.

5.2 Drill a small hole at the specified coordinates on the skull.

6 Viral Injection (Optional):

6.1 Attach a capillary needle to the Quintessential Stereotaxic Injector.

6.2 Slowly lower the needle to the target brain region.

6.3 Inject the viral vector at a controlled rate.

6.4 Leave the needle in place for 5 minutes post-injection to prevent backflow.

7 6-OHDA Injection:

7.1 Prepare a 6-OHDA solution (0.6 µg/µL in 0.02% ascorbate).

7.2 Use the following stereotaxic coordinates for injection into the medial forebrain bundle of the right hemisphere: AP: −1.2 mm, ML: +1.25 mm, DV: −4.75 mm (relative to bregma and dural surface).

7.3 Inject 1 µL of 6-OHDA solution at a controlled rate.

7.4 Leave the needle in place for 5 minutes post-injection to minimize backflow.

7.5 Slowly retract the needle from the brain.

8 Needle Retraction and Closure:

8.1 Close the incision with sutures or surgical glue.

8.2 Clean the surgical area with sterile saline.

Postoperative Care

9 Immediate Postoperative Care:

9.1 Administer Carprofen (5 mg/kg, subcutaneous) as a postoperative analgesic.

9.2 Place the mouse in a warm recovery area and monitor until fully awake.

10 Continued Analgesia and Monitoring:

10.1 Provide a second dose of Carprofen 18-24 hours after surgery.

10.2 Monitor the animal daily for signs of pain, distress, or infection.

10.3 Record body weight and behavior to assess recovery.

11 Special Postoperative Care for 6-OHDA Lesion Model:

11.1 Provide sweetened milk (1:3 dilution in tap water) in the home cage to support recovery and maintain body weight.

11.2 Monitor the animal daily for one week for any signs of distress or motor deficits.

11.3 Histological or behavioral assessments should be conducted at least two weeks post-injection to evaluate the efficacy of the lesion.