

Jun 30, 2022

# Standard Operating Procedure: Mouse Stereotaxic Intracranial Injection Surgery

DOI

[dx.doi.org/10.17504/protocols.io.rm7vzye28lx1/v1](https://dx.doi.org/10.17504/protocols.io.rm7vzye28lx1/v1)

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**Protocol Citation:** Hong-Yuan Chu 2022. Standard Operating Procedure: Mouse Stereotaxic Intracranial Injection Surgery. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.rm7vzye28lx1/v1>

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**Protocol status:** Working

We use this protocol and it's working

**Created:** June 27, 2022

**Last Modified:** May 31, 2024

**Protocol Integer ID:** 65426

**Keywords:** Stereotaxic surgery, Intracranial Injection, Rodents, ASAPCRN, mouse stereotaxic intracranial injection surgery the intent, mouse stereotaxic intracranial injection surgery, stereotaxic intracranial injection surgery, procedures for mouse, standard operating procedure, procedure, sop, injection, mouse

**Funders Acknowledgements:**

**ASAP**

Grant ID: ASAP-020572

**Department of Defense**

Grant ID: W81XWH2110943

## Abstract

The intent of this Standard Operating Procedure (SOP) is to describe procedures for mouse stereotaxic intracranial injection surgery.

## Attachments



[h78ebu5cf.docx](#)

21KB

## Guidelines

**RESPONSIBILITY:**

Principal investigator (PI), their staff, or any individual performing stereotaxic surgery on rodents or assisting in those procedures.

## Materials

### **MATERIALS:**

- Anesthetics (e.g. isoflurane) and induction chamber.
  - 1.2 charcoal filter canisters for isoflurane output
  2. Necessary tubing to connect equipment
- Stereotaxic surgery frame
- Drill with 0.5 mm drill bit, power supply, and foot pedal
- Warming pad, anal probe, and power supply
- Injector
- Electric clipper
- Non-steroidal analgesic (e.g. carprofen)
- Sterile 0.9% saline
- Antiseptic solution for skin (e.g., povidone-iodine solution and 70% ethanol)
- Sterile ophthalmic ointment
- Sterile surgical instruments
  1. Scalpel
  2. Blunt Forceps
  3. Dumont forceps
  4. Fine Scissors
  5. Needle holder
- Sterile swabs and gelfoam spears
- Sterile drapes
- Sterile Hamilton syringe (1700 Hmltn, 10uL)
- Suture material

## Troubleshooting

## Surgical and Station Preparation:

- 1 Print the surgery documents and prepare them from Chu Lab SharePoint.
- 2 Prepare solutions needed for target injections according Chu Lab Inventory and SOPs from the Chu Lab SharePoint. Place solutions on ice, if applicable.
- 3 Autoclave the necessary tools to prior to surgery.
- 4 Disinfection surgery station and place down a benchpad.
- 5 Setup the necessary equipment including:
  - Stereotaxic frame
  - Injector
  - Warming pad
  - Drill
  - Induction Chamber
- 6 Check the isoflurane levels. Levels visible on the front of the vaporizer.
- 7 Designate a sterile area on the working surface for the sterile material (instruments, suture material, gauze, etc.).
- 8 Clean the Hamilton syringe with sterile water, then 70% ethanol then sterile water again.

### Note

Note: Make sure it is not clogged and that the plunger can move up and down smoothly.
- 8.1 Check the needle is straight before beginning and replace if it appears bent.
- 8.2 Secure to needle to stereotaxic frame and adjust out of the way to avoid contact with needle.



## Pre-operative Procedures:

9

### Note

Note: Perform pre-operative procedures at a safe distance from the surgical environment in order to prevent contamination with hair.

Anesthetize the animal in induction chamber.

- 9.1 Open valves to oxygen tank and adjust to a flow of  1 L per  00:01:00 . Adjust the level of vaporizer to 3 percent isoflurane in oxygen and wait for the cessation of movement.


1m

- 10 Shave top of the head and remove hair.

### Note

This is often done over trash can or away from surgical setup.

Return animal to induction chamber if need before next step.

- 11 Secure animal in the stereotaxic frame. Once nose cone is in place, close valve to chamber and open valve to nose cone and reduce vaporizer to 1.5-2 percent isoflurane in oxygen.
- 12 Apply ophthalmic ointment in both eyes to prevent corneal desiccation. Reapply as needed.
- 13 Place anal probe underneath body to regulate temperature. Heating system prove setpoint is  36.9 °C .

## Surgical Procedures:

- 14 Using 70% ethanol on a sterile cotton swab in a circular motion, sterilize surgery site from the center of the surgical site to the exterior.
- 14.1 Then apply povidone-iodine solution using cotton swabs in a circular motion from the center of the surgical site to the exterior. Repeat this process for a second time.
- 15 Expose the cranium by making a surgical anterior-posterior incision with a scalpel blade and reflect the skin.



16 Use scope to locate bregma and lambda.

16.1 They will become more prominent over time and hydrogen peroxide solution can be applied to the skull to facilitate identification.

17 Position needle directly over the intersection with the coronal and sagittal sutures to locate bregma.

17.1 Lower needle until it just touches the surface of the skull.

17.2 Record the A-P, M-L, and D-V coordinates of Bregma.

**Note**

Note: For assistance, refer to photo reference on surgery document.

18 Repeat step 17 at the intersection of the lambdoid and sagittal sutures to locate Lambda.

18.1 Record the A-P, M-L and D-V coordinates of Lambda.

19 Adjust the level of the skull until there is less than a 50um D-V difference between Bregma and Lambda, and less than a 50um D-V difference between point +2 and -2 mm M-L to Bregma.

20 Calculate target coordinates for injection site using leveled bregma coordinates.

21 Move needle to target site and mark site using a pencil.

22 Raise needle and, using the hand-held drill, make a single burr-hole in the skull at the injection site. Clean hole of any blood or debris using sterile saline and gelfoam sponges.

23 Draw up necessary volume of solution into the syringe.

**Note**

Lower plunger until a drop of solution is visible at the end of the needle to ensure the solution is at the bottom of the needle and there are no air bubbles.


24 Remeasure the D-V coordinate at the dura and calculate the target D-V coordinate.

25 Gradually lower the injection needle gradually to target D-V and wait  00:01:00 .



1m

26 Check that settings on the injector are correct:



**Syringe Settings:**


- 1700 Hmltn  10  $\mu$ L 0.460mm.
- Adjust volume and speed depending on injection.
- Check injector is set to infusion.

27 Press START button on injector to begin injection.

28 Once injection is done, wait  00:03:00 -  00:05:00 before retracting needle to avoid backflow.

18m

- For  1 mL of 6-OHDA, wait  00:10:00 .

29 Gradually withdraw needle 500  $\mu$ m and wait  00:01:00 . Repeat. Continue to withdraw needle until completely removed from the brain.

1m

30 Repeat the procedure if more than one injection is required.

**Note**

If performing a bilateral set of injections, check that the needle is not blocked before performing the second injection.

31 Suture skin with sterile suture (polyamide-nylon, PDS, Vicryl; size: 3-0 or 5-0) and sterilize skin with betadine.

**Post-Operative Procedures:**



- 32 Administer carprofen (5mg/kg, s.c.).
- 33 Remove animal from stereotaxic frame and place animal into a cage on a warming pad.
  - 33.1 Observe until animal is awaking and regained righting reflexes.
- 34 Document surgery on a surgical procedure cage card and return cage to appropriate room in the vivarium.

**Note**

If injecting a biohazard (e.g. AAV), the animal must be housed in the CH1 biohazard room for 🕒 72:00:00 with appropriate labeling.

- 35 Administer Carprofen (5mg/kg, s.c.) the next day.

**Note**

If mouse was injected with 6-OHDA, supplement with additional food (e.g. yogurt drops) to avoid excessive weight loss.

- 36 Continue daily observations for 3 days post-operation and record on the back of the surgical procedure cage card.
  - 36.1 Extend observation period as necessary to ensure a healthy recovery.