MAR 20, 2024

DOI:
dx.doi.org/10.17504/protocols.io.81wgbx373lpk/v1

Protocol Citation: madalynn.erb 2024. Mouse stereotaxic surgery. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbx373lpk/v1

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

Created: Feb 09, 2024

Mouse stereotaxic surgery

madalynn.erb[1]

[1]Van Andel Institute

ASAP Collaborative Research Network | Team Lee

Jane Balster
ASAP - Team Lee

ABSTRACT

This protocol details the mouse stereotaxic surgery.

ATTACHMENTS

1003-2591.docx

Last Modified: Mar 20, 2024

PROTOCOL integer ID: 95133

Keywords: ASAPCRN

Funders Acknowledgement:
Madalynn Erb
Grant ID: ASAP000592

MATERIALS

Materials:

- Anesthesia : Inhalant Isoflurane

1. Induction: 3.0 - 3.5%
2. Maintenance: 2.0 – 2.5%

- Analgesia :

1. Marcaine (Local)
2. Ethiqa XR / buprenorphine extended-release (Systemic)

- Sterile 0.9% saline
- Sterile ophthalmic ointment
- Electric hair clippers
- 70% ethanol
- 3% Hydrogen peroxide
- Motorized stereotaxic frame
- Heating pad
- Sterile surgical instruments
- Sterile gauze and swabs
- Surgical drill
- Hamilton syringe
- Steel needle
- Surgical tubing
- Pump
- Surgical glue

Procedure

3d 0h 6m 30s

1 Turn on the heating pad to and set to 36.9 °C .

2 Weigh mouse and record weight before starting surgery.

3 Place mouse in isoflurane chamber (3% isoflurane).

3.1 Record time of anesthesia.

4 After mouse is fully anesthetized, use hair clippers to shave head.

5 Place mouse back into isoflurane chamber (3% isoflurane) until fully anesthetized.

6 Transfer mouse onto stereotaxic frame and place the mouth / nose into nose cone with isoflurane.

6.1 Turn down isoflurane to 2%.

7 Apply sterile ophthalmic ointment to eyes using a sterile swab (this will prevent desiccation).

8 Use ear bars to secure mouse in stereotaxic frame ensuring head is level in all directions.

9 Using a sterile swab apply 70% EtOH to the scalp.

10 Using a sterile swab, apply povidone-iodine solution to the scalp.

10.1 Wait for povidone-iodine solution to dry before making surgical incision.

11 Perform a subcutaneous injection of Ethiqa XR (buprenorphine extended-release) into the leg.

11.1 Use 0.05 mL per 20 g mouse.

12 Inject 30 µL of Marcaine in 2-3 locations underneath the scalp near the incision site. This is a local analgesic.

12.1 Wait 30 seconds - 00:01:00 for the Marcaine to diffuse before performing incision. 1m

13 Using a sterile scalpel, make a surgical incision to expose the skull.

13.1 Minimize the size of the incision as much as possible.

Note

You will need to see bregma and have access to the injection site. For substantia nigra injections this will be located at the caudal region of the skull.

14 Position your injection needle at bregma and save location in the AP and XY axis.

15 Enter and save injection coordinates into the motorized stereotaxic frame.

15.1 Coordinates for right substantia nigra

- Anterior-posterior (AP): -2.9 mm
- Medial-lateral (ML): -1.3 mm
- Dorso-ventral (DV): -4.2

16 Raise the needle away from the skull slightly and move to AP and ML injection coordinates.

17 Slowly lower the needle to touch the skull. Raise the needle and drill a small hole where the needle touched the skull.

17.1 Slowly drill through the skull keeping the drill shallow enough to not damage brain tissue.

18 Once the hole is drilled lower the needle to the surface of the brain ensuring that the needle is not deflected by the skull.

Note

The needle should be completely straight.

19 Raise the needle 30 mm - 40 mm providing space to flush the needle and load with virus.

19.1 Flush the needle with sterile Room temperature H_2O.
- Cover the mouse's head with sterile gauze to absorb the H_2O.

19.2 Draw up a 1 µL air bubble.

19.3 Load needle with 2.5 µL of virus.
- Slowly draw up virus and watch the air / liquid interface to determine volume.
- Needle / tubing should be marked with 1 µL intervals using a sharpie to help with this step.

20 Move the prepared needle down to the surface of the brain. Move the needle to the desired DV coordinate at a slow speed (250µm / sec).

21 Wait 00:00:30 and then start the virus injections at 0.2 µL / min. 30s

22 After the injection is finished wait for 00:05:00, leaving the needle in place and allowing the virus to diffuse away from injection site. 5m

23 Remove the needle at slow speed (250 µm / sec).

24 Move the needle up away from the skull and then release it and rotate it out of the way.

25 Close the incision site using sterile forceps and surgical glue.

26 Administer 0.5 mL sterile saline solution subcutaneously to avoid dehydration.

27 Place the mouse back into the home cage and monitor recovery.

27.1 Record time at recovery.

28 Monitor post operation recover for 72:00:00 and record any observations of pain or distress onto surgery cards. 3d