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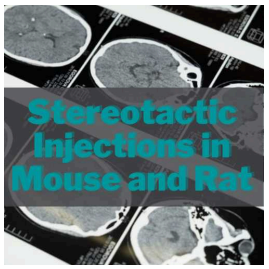
Stereotactic Injections in Mouse and Rat



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

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Abstract

This protocol outlines procedures for Stereotactic Injections in Mouse and Rat.

Attachments

PDF

STEREOTACTIC_INJECTI
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193KB

Materials

Anesthesia Reagents Needed and Preparation

- **Rats** (60 mg/kg Ketamine ; 0.4 mg/kg medetomidine):
  - 0.60 ml Nimatek + 0.40 ml Domitor + 1.00 ml Saline = 2 ml anesthetic cocktail
  - Use: *0.15 ml / 100 g of body weight*
  - Administration route: IP
  - If the injection is given properly, the rat will sleep in 2 minutes
- **Mouse** (75 mg/kg ketamine ; 1 mg/kg medetomidine):
  - 0.15 ml Nimatek + 0.20 ml Domitor + 1.65 ml Saline = 2 ml anesthetic cocktail
  - Use: *0.1 ml / 10 g of body weight*
  - Administration route: IP
  - If the injection is given properly, the mouse will sleep in 2 minutes

Reversal of Anesthesia Reagents Needed and Preparation

- **Rats**:
  - 1.0 ml Antisedan + 4.0 ml Saline = 5 ml antidote
  - Use: *0.2 ml / 100g of body weight*
  - Administration route: IP
- **Mouse**:
  - 0.1 ml Antisedan + 9.9 ml Saline = 10 ml antidote
  - Use: *0.1 ml / 10 g of body weight*
  - Administration route: IP

Analgesia (Post - operative analgesia)

- Dilute Vetergesic 10x
- **Rats**: 150 µl / 100 g of body weight
- **Mice**: 30 µl / 10 g of body weight
- Analgesic effect will last 8-12 hours
- Put liquid Xylocaïne drops upon the skull if you notice that the animal is suffering pain.

Materials Needed

- Big scissors (for the hair), small scissors, curved forceps, needle holder, spatula, scalpel holder
- Xylocaïne 2%, joodalcohol, Vidisic
- Chip(holder)
- Magnifier, blade nr 10, wire 3-0 (Rat) / 4-0 (Mouse)
- 10 ml syringe + pink needle, small tissues (sterile)
- Small pots to rinse the Hamilton syringe (RBS, ETOH, PBS, PBS, AD)
- 4× 1 ml syringe + needle (anesthesia, reversed, painkiller, +1)
- Hamilton syringe + needle
- Pipet + tips + eppendorfs

Troubleshooting

Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Procedure

6m

1 Remove hair and put [M] 2 % Xylocaïne gel on top of the head and into the ears.

2 Put Vidisic on the eyes.

3 Put a chip under the skin to mark the animal.

4 Fix the animal into the stereotactic apparatus (use the mouse adaptor for mouse and only the ear clamps for rat). Put a tissue over the animal to keep it warm during surgery.

5 Make sure that the left and right side of the skull is positioned as straight as possible (ears).

6 Use [M] 1 % joodalcohol to clean the top of the head and make an incision with a scalpel.

7 Clean the skull with a spatula and saline and let dry until bregma and lambda are clearly visible.

8 Check volume and injection speed of the pump, rinse Hamilton syringe with RBS, ETOH and PBS. (Coat the syringe by taking a full syringe of vector and discard in eppendorf). Put vector in the syringe (± 1.5 µl more than you want to inject) and make sure there are no air bubbles.

9 Make sure bregma and lambda have the same height, correct the position of the head if there's a difference of more than 0.02cm.

10 Put the needle at the right position using bregma as a reference (find the right coordinates using the stereotactic atlas of mouse or rat).

11 Drill a small hole into the skull at this position until the dura mater is visible.

12 Make a small hole into the dura mater using a thin needle.

13 Put the Hamilton needle at the right position (go down slowly to prevent tissue damage) and wait for 00:01:00 . 1m

14 Inject vector at max. 0.25 µl/min.

15 Wait 00:05:00 after injection in order to let the vector diffuse into the brain. 5m

16 Remove the needle slowly.

17 Close the skin and disinfect with joodalcohol.

18 Rinse Hamilton syringe with RBS, ETOH, PBS and AD.