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Stereotaxic injection of viral vectors

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ABSTRACT

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MATERIALS TEXT

Reagents

- Ketamine
- Bupivacaine HCl local anesthetic
- Alcohol prep wipes
- Iodine prep wipes
- Sterile ocular lubricant
- Filtered HBS
- Virus (AAV)

Instruments and Materials

- Kopf model 1900 Sterotaxic alignment instrument
- Autoclaved fine surgery tools
- Sterile towel drapes
- Laboratory scale
- Electric hair shaver
- Sterile tip cotton swabs
- 1 ml needles and syringes for IP injection of anesthetics and analgesics
- Drill
- Hamilton syringes (1 µL Catalog #65458-01)
- Surgical sutures
- Temperature-regulated heat pad

Sterotaxic surgery steps

1 Clean the Hamilton Syringe with 70% and then with distilled water.

2 Calibrate Kopf frame according to the instructions.

3 Position the heat pad covered with the sterile towel drapes next to the stereotaxic instrument. Cover the working area of the bench with the towel drapes.

4 Anesthetize the animal with 3-4% Isoflurane.

5 Weight the animal and deliver ketamine (100 mg/kg) via intraperitoneal injection. The animal should reach surgical anesthesia within 5–10 min and should not respond to a light pinch to the hind paw or tail.

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6 Shave the fur off the top of the animal's skull.

7 Place the animal in the stereotaxic instrument. To do so, carefully place one ear bar in the ear canal, secure the bar, and hold the animal in place as the other ear bar is placed and secured. The animal should not be able to move laterally.

8 Next, secure the mouth in the incisor adapter of the stereotaxic instrument, taking care that the tongue is not pinched in the adapter or blocking the airway. The nose clamp can be gently tightened to firmly secure the animal's head in position.

9 Cover the anesthetized animal's eyes with sterile ocular lubricant to keep them moist during the surgery.

10 Apply local anesthetic Buprivacaine under the head skin.

11 Clean the surface of the skin with alcohol and Iodine prep wipes.

12 Make a midline incision to the top of the animal's skull with small surgical scissors or a scalpel.

13 Clean skull with cotton swabs.

14 Install the Centering Scope. While sighting down through the scope adjust and focus the head using the lateral, A-P and vertical shift knobs on the head holder. Scope is already positioned, now you are adjusting the head accordingly.

15 Find bregma. Once Bregma is focused and aligned with the cross, measure the distance between Bregma and Lambda. At this point the DRO should read (0, 0)

16 By looking through the scope, move the Y knob to Lambda. Read the DRO Y-axis display. What

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Y displays will be divided by 2.

17 The reason of the previous step is to set the distance between Bregma and Lambda on the Alignment Indicator. Install the Indicator into the holder and check the flatness of the skull.

18 Install again the Centering Scope and bring it down into focus. Do this by returning the Z-axis back down to zero. Since you positioned Y between bregma and lambda, alignment will be visible at this position.

19 In this time, you need to line Bregma and Lambda. To do so, use Sagittal Alignment Knob. Go back, find bregma and zero everything. Remove the Centering Scope and install the drill.

20 Set your X-Y coordinates.
ØCoordinates for SNc injection:
AP: -3.1 / -2.7
ML: 1.15 / 1.45
DV: 4.3 / 4.1
ØCoordinates for PPN injection:
AP: -4.5
ML: 1.1
DV: 3.75 / 3.50
ØCoordinates for STN injection:
AP: -1.9
ML: 1.6
DV: 4.6 / 4.4

21 Remove the drill and install the Hamilton syringe.

22 Load the viral solution.

23 Find bregma with the syringe needle and zero everything again

24 Find your coordinates

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25 Deliver virus by 100 nl/min speed

26 After injections, wait for 10 minutes for the virus to diffuse through the tissue. Then come up with 1 cm and wait for another 2 minutes

27 Remove the syringe very carefully

28 Close the incision via surgical suture

29 Make subcutaneous injection of Meloxicam the day of the surgery and after 24 hours

30 Remove the animal from the stereotaxic frame, return him back to the cage and monitor the animal's recovery

31 Clean the Hamilton syringe with 70% ethanol and distilled water

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