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VTA Surgery Protocol

In 1 collection

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ABSTRACT

This protocol details the VTA surgery procedures used to infect VTA dopamine neurons with virus and implant various cannulas and fibers intracranially.

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Setup:

1 Wipe down the whole surgical area and the stereotax (Kopf Model 1900) with 100% ethanol.

2 Prep your sterile surface with

- surgical tools (scissors, fine forceps, graefe forceps),
- surgical Eyespears,
- sterile cotton gauze pads,
- sterile cotton tipped applicators,
- a scalpel,
- and something to mark the skull (ex., a pen or fine-tip needle).

3 Center the stereotax.

3.1 Place the Centering Height Gauge (Kopf Model 1900-51) on the stereotax base, rotate it to the ‘plus’ position.

3.2 Attach the Centering Scope (Kopf Model 1915) to the stereotax arm.

3.3 Center the scope crosshairs on the tool.

3.4 Zero the X and Y coordinates on the Digital Display Console.

4 Calibrate the Stereotaxic Alignment Indicator (Kopf Model 1905).

4.1 Attach the Alignment Indicator to the stereotax arm.

4.2 Rotate the Centering Gauge 45 degrees to an ‘X’ orientation.

4.3 You want the two dials to both hit zero at the same time when lowering the arm in the Z direction. Avoid coming down on the Centering Gauge grooves – aim for the flat area adjacent to the groove instead. If the two dials do not hit zero at the same time, rotate the dials until they are perfectly zeroed.

5 Prepare the Narishige injection pump (MO-10).

5.1 Prepare the injection needle by cutting the sealed tip off a pulled capillary (Drummond Scientific Company Wiretrol II, pulled with Sutter Instrument P-97). The needle needs to be long enough to access the VTA (>5mm).

5.2 Backfill the injection needle with red mineral oil, being careful to avoid introducing any bubbles into the needle.

5.3 Loosen the hex screw on the injection pump slightly.

5.4 Slide the needle over the metal injection plunger, through the chuck, stopping when the needle is just barely protruding out of the rear of the chuck.

5.5 Tighten the hex screw with an Allen wrench (using the shorter moment arm so as not to overtighten the screw – overtightening can crack the capillary).

6 Prepare your virus (dilute in hyperosmotic PBS to desired titer) and any implants being used.

Begin surgery:

30s

7 Weigh the mouse, calculate the amount of Buprenorphine SR to inject, and prepare a .3mL insulin syringe with the correct volume.

8 Prepare the isofluorane chamber: oxygen flow rate to 1 mL, with 3% isofluorane.

9 After a minute or two to preload the chamber, put the mouse in.

10 Once mouse is down (~1 breath per second, no tail pinch response), set valves so that gas is directed to the stereotax and drop isofluorane to 2% and oxygen to 0.6 mL .

11 Place mouse in ear bars.

11.1 Bite bar goes into its mouth, with the top teeth secured through the hole in the bite bar and the tongue pushed out to the side so it is not occluding the throat.

11.2 Nose cone loosely in place. Tight enough to prevent mouse from coming off the bite bar, loose enough to allow movement of the head for positioning.

11.3 Place the ear bars slightly above the ear canals in a divot on the skull, such that the skull is centered between the two bars, and tighten until the mouse's skull is stabilized.

11.4 Tighten the nose cone on the snout.

12 Using a cotton tipped applicator, apply Puralube to the whiskers to hold them down and out of the way.

13 Using a cotton tipped applicator, apply Nair to the scalp, working against the grain of the fur.

Note

Be careful to avoid the eyes.

13.1 After ~ 00:00:30 , wipe off the Nair with cotton tipped applicators – the fur should come off easily as well. Repeat until a circular area on top of the skull has been cleared. 30s

14 Alternate sterilizing the cleared skin with betadine (cotton tipped applicator) and 70% ethanol (gauze pad) (3x each).

15 Using a cotton tipped applicator, cover the eyes with Puralube.

16 Inject the Buprenorphine SR subcutaneously.

17 Remove non-sterile gloves, and put on sterile surgical gloves.

18 Use forceps to lift the scalp, and use scissors to remove the scalp. You should have a teardrop shape of exposed skull with both bregma and lambda visible. Remove as much of the periosteum as possible using the forceps and scissors.

19 Use a cotton tipped applicator dipped in hydrogen peroxide to remove the remaining periosteum, being careful to avoid the skin around the edges of the incision.

20 Apply Bupivacaine 0.25% (a local analgesic) to the skin around the edges of the incision.

21 For electrode implants only: insert two small screws into the skull for grounding.

21.1 Using a carbide drill bit (Alpen Carbide Instruments R1001/4c), drill two craniotomies, one posterior to lambda (at the midline to minimize bleeding) and one over the olfactory bulb (anterior and lateral to bregma – stay away from the midline to minimize bleeding).

21.2 Place a No. 60 jobber drill bit in each craniotomy and rotate it gently clockwise to form tracks.

21.3 Screw a screw (P1-Tech 00-96X, 1/16, 1.6mm) into each craniotomy, just far enough that it is stable and cannot be pulled out with forceps, but not so far as to cause brain tissue damage.

22 Use the Centering Scope to view and mark bregma.

23 Level the skull at bregma using the Alignment Indicator in both the M/L and A/P directions.

24 Mark your drilling coordinates using the fine-tipped pen/needle.

25 Begin drilling, using a carbide drill bit (Alpen Carbide Instruments R1001/4c), one site at a time, stopping before penetrating the brain tissue. Use fine tip forceps to remove any bone fragments from the injection holes, and surgical spears to stop bleeding as needed.

Virus injection:

26 Load the Narishige injection needle with virus.

26.1 Attach the Narishige to the stereotax arm, and place Parafilm (sterile side up) on a flat reachable surface such as the ear bars.

26.2 Thoroughly mix virus aliquot with a pipette, then place ~ 2 µL of virus as a bubble on the parafilm.

26.3 Lower the needle into the bubble while extracting a small amount to ensure no air bubble between the mineral oil and the virus.

26.4 Slowly backfill the Narishige, loading the needle with 0.8 µL - 1 µL of virus.

27 Center the injection needle on bregma and zero the ‘X’ and ‘Y’ coordinates on the Display Console.

28 Move to -3.2 undetermined and zero the ‘Z’ coordinate with the needle touching the skull at the midline.

29 Move the needle to your first injection site (-0.5 mL).

29.1 Lower needle to lowest DV site (-5.0DV) and slowly inject 100 undetermined (this should take at least 30s), then wait 2 mins .

29.2 Raise the needle to the higher DV site (-4.5DV) and slowly inject 100 undetermined , then wait 3 mins.

29.3 Raise the needle out of the brain, and repeat steps for the next injection site (0.5 mL).

30 Fill any craniotomies that will not be used for an implant with bone wax.

31 Every 1 hour of surgery time that passes, give sterile saline with 5% dextrose (1 mL subcutaneously).

32 If not adding any implants (for brain slice electrophysiology), suture the skin closed, using Vetbond as necessary to close incision, and end surgery. Else, continue to next step.

Implant setup:

20s

33 Thoroughly scratch the skull in a crosshairs pattern with the scalpel.

34 Cover the skull with Optibond (being careful to not cover craniotomies or touch the skin) and set with 00:00:20 of UV light. 20s

35 Put bupivacaine on the edge of the skin to re-moisten.

Cannula + electrode implant:

36 Put dental cement on the base of the headbar (custom design), and then place headbar on the top of the skull (headbar will not fit over electrode array, must be placed first).

37 **Electrode implant:**

37.1 Clean cannula of electrode array (Innovative Neurophysiology, Inc.; 23µm Tungsten Electrodes, 16/bundle; 0.008” silver ground wire) with 100% ethanol, then place in stereotaxic cannula holder (Kopf Model 1966) and attach to the stereotax arm.

37.2 Wrap the array’s ground wire tightly around both screws, wrapping around each 2-3 times to ensure good grounding.

37.3 Lower the array cannula to the skull surface next to the craniotomy (-3.2mm AP, -0.5mm ML) and zero ‘Z’.

37.4 Lower the implant slowly, ~ 0.5 undetermined at a time with 2 minutes of wait between each chunk – until it reaches -4.0mm DV.

37.5 Put a small dab of superglue around the base, and coat with dental cement, being careful to not cover the cannula craniotomy, and wait until dry before removing the cannula holder.

38 **Cannula implant:**

38.1 Clean unilateral guide cannula (P1 Tech, mini, cut to 13.5mm) and dummy (no projection) by soaking in 100% ethanol.

38.2 Dry with air can, insert dummy into guide cannula, then place in stereotaxic cannula holder and attach to the stereotax arm.

38.3 Lower the cannula to touch the skull surface next to the craniotomy (-3.2mm AP, -1.3mm ML) and zero ‘Z’.

38.4 Lower the implant slowly, ~ 0.5 undetermined at a time with 2 minutes of wait between each chunk, until it reaches -4.0mm DV.

38.5 Put a small dab of superglue around the base, and coat with dental cement, and wait until dry before removing the cannula holder.

Bilateral cannula implant:

39 **Cannula implant:**

39.1 Clean bilateral guide cannula (P1 Tech, 1mm spacing, cut to 4mm) and dummy (.25mm projection) by soaking in 100% ethanol.

39.2 Dry with air can, insert dummy into guide cannula, then place in stereotaxic cannula holder (Kopf Model 1966) and attach to the stereotax arm.

39.3 Lower the cannula to touch the skull surface next to the craniotomies (-3.2mm AP, +/-0.5 mm ML) and zero ‘Z’.

39.4 Lower the implant slowly, ~.5mm at a time with 2 minutes of wait between each chunk, until it reaches -3.75 mm DV.

39.5 Put a small dab of superglue around the base, and coat with dental cement, and wait until dry before removing the cannula holder.

40 Put dental cement on the base of the headbar (custom design), and then place headbar on the top of the skull.

Cannula + fiber optic implant:

41 **Fiber implant:**

41.1 Clean fiber (Doric Lenses, MFC_400/430-0.66_5mm_MF1.25_FLT) with 100% ethanol.

41.2 Dry with air can and place in stereotaxic cannula holder (Kopf Model 1966, holding fiber cap), and attach to the stereotax arm.

41.3 Angle skull (via u-bar tilt) to a 6 degree angle outwards from the left hemisphere.

41.4 Lower the fiber to touch the skull surface next to the craniotomy (-3.2mm AP, -0.952mm ML) and zero 'Z'.

> Note
>
> ML is adjusted for the 6 degree angle for a final fiber tip ML of -0.55 mL .

41.5 Lower the implant slowly, ~ 0.5 undetermined at a time with 2 minutes of wait between each chunk, until it reaches -4.324 undetermined DV.

Note

DV is adjusted for the 6 degree angle for a final fiber tip DV of -4.33 undetermined DV.

41.6 Put a small dab of superglue around the base, and coat with dental cement, being careful to not cover the cannula craniotomy, and wait until fully dry before removing the cannula holder.

41.7 Carefully remove the fiber cap from the fiber.

42 Cannula implant:

42.1 Clean unilateral guide cannula (P1 Tech, mini, cut to 7mm) and dummy (no projection) by soaking in 100% ethanol.

42.2 Dry with air can, insert dummy into guide cannula, then place in stereotaxic cannula holder and attach to the stereotax arm.

42.3 Angle skull (via a u-bar tilt) to a 10 degree angle outwards from the right hemisphere.

42.4 Lower the cannula to touch the skull surface next to the craniotomy (-3.2mm AP, +0.749 mm ML) and zero ‘Z’.

Note

ML is adjusted for the 10 degree angle for a final fiber tip ML of 0 mm ML.

42.5 Lower the implant slowly, ~ 0.5 undetermined at a time with 2 minutes of wait between each chunk, until it reaches -4.316 undetermined DV.

Note

DV is adjusted for the 10 degree angle for a final fiber tip DV of -4.25 undetermined DV.

42.6 Put a small dab of superglue around the base, and coat with dental cement, and wait until dry before removing the cannula holder.

43 Put dental cement on the base of the headbar (custom design), and then place headbar on the top of the skull.

Complete implant:

44 Give saline with 5% dextrose (1mL subcutaneously) if another hour of surgery time has passed.

45 Fill headbar with dental cement, ensuring no skull is left uncovered. Use Vetbond to glue skin to the side of the headbar if necessary.

45.1 If later headfixing, place a small wooden dowel in the headcap and stabilize with dental cement to make headfixing easier.

46 Wait for dental cement to dry, then add cannula dust cap, headcap, etc to the implants as needed.

End surgery:

47 Turn isofluorane off, and leave mouse under 0.6 mL oxygen for a few minutes until it begins to wake.

48 Weigh the mouse post-surgery and leave in a cage to recover.

49 Perform the necessary post-operative care procedures on subsequent days.