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| SOP No: | AHP 74 |
| SUBJECT: | Intracerebroventricular cannula implantation in the rat |
| POLICY: | This procedure may only be performed by operators skilled in the technique. Surgery must be performed under aseptic conditions |
| PRECAUTIONS: | Surgical gloves, eye protection, long-sleeved gown, closed in shoes. All instruments and materials (including catheter) must be sterilized before use. |
| EQUIPMENT: | Anesthetic Analgesic for 24hr cover stereotaxic frame rats brain atlas (i.e. Paxinos and Watson, 2005) nickel alloy jeweller's screws (1.4mm x 3.5mm) low speed drill (1.4mm diam) low-temperature Diathermy pulse-oximeter lacrilube eye ointment stainless steel cannula (9.0mm length, 22 Gauge, inner diameter 0.39mm, outer diameter 0.71mm with a nylon pedestal) dummy cannula (for closer of the cannula) (9.5mm length, 0.36mm diameter stainless steel wire with nylon screw cap) internal (injection) needle, with attached, saline filled polyethylene line 10µl Hamilton syringe forceps, small curved small and large hemostats small and large forceps scalpel blade (Size 11) surgical silk (2.0) dental cement (Vertex) disposables (Cotton swabs and tips, syringes (3.0ml), hypodermic needles (26G)) |

- PROCEDURE:**
- 1. Record weight of animal on experimental card.**
 - 2. Anaesthetise rat**
 - 3. Shave head region and disinfect with chlorhexidine (1%), ethanol (70%) and Iso-betadine (10% polyvidon iodine) from the centre of the site to the periphery.**
 - 4. Make single midline incision on scalp between eyes and nape of neck.**
 - 5. Reflect skin and connective tissue overlying skull by pushing it laterally using blunt edge of scalpel.**
 - 6. Stop all bleeding using diathermy.**
 - 7. Drill two burr holes bilaterally (1.4mm diameter) in the frontal bone (approx. 2mm anterior to the coronal suture) and two in the parietal bone (approx. 2mm anterior lambdoid suture) using a low speed electrical drill**
 - 8. Insert anchor screws (no more than 2.5 rotations)**
 - 9. The animal is fixated in the stereotaxic frame by means of ear bars.**
 - 10. Prime injecting line and put an air bubble in it. Insert the injecting needle into the guide cannula and attach to stereotaxic frame.**
 - 11. Move the cannula -1.5 mm lateral and 1.0 mm posterior from bregma and mark it on the skull with a pencil (unless a digital frame is used).**
 - 12. Move the cannula up, drill a bit and verify position by moving the cannula back down, then complete drilling by carefully drilling through to the skull.**
 - 13. Gently pierce the dura with a needle, wire away any blood and lower the cannula into the hole, where it touches the dura (usually ~1 mm below the surface of the skull).**
 - 14. Lower the cannula into the hole, to 3.0 mm below the dural surface.**
 - 15. Between the depth of 3.0 and 3.5 mm, check the injecting line for drawback every 0.1 mm.**
 - 16. When drawback is observed, the cannula is appropriately placed.**
 - 17. If no drawback is seen in this range, move to a dorsoventral coordinate of 3.4 mm below dural surface and implant there.**
 - 18. Ensure skull is very dry (use small amount of hydrogen peroxide on end of swab).**
 - 19. Make up dental cement and mould around cannula and anchor screws, making sure none is attached to skin.**
 - 20. After the cement has dried make a purse-string suture around the cement, allowing the skin to hug the cap.**

- 21. Remove animal from frame, take out injecting needle and place dummy into cannula.**
- 22. Administer analgesic for 24h.**

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REVISED:

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