# LAB_081 Chronic Cannula Implantation in the Brain in Rodents (Expiry Jan 2027)

## I. OBJECTIVE

The intent of this procedure is to describe the surgical procedures for implanting a chronically placed cannula into the brain tissue or intracerebroventricular. This allows for easy and repeated access to inject into the brain or cerebrospinal fluid of a rodent.

## II. COMMENTS / RECOMMENDATIONS

- Users must keep monitoring records, which includes anaesthesia monitoring records, surgical records and post operative monitoring scoresheets (example templates can be obtained by contacting the UQBR Veterinarians or Animal Ethics Unit Veterinary Officer).
- Any associated experimental compounds or medications (including your anaesthetic protocol) must be detailed within the Animal Ethics Committee (AEC) application.
- Wherever possible, active heating (e.g. a heat mat) should be used at all times, including while the rodent is in the stereotactic device, to help maintain normal body temperature of the anaesthetised rodent.
- Considerations for the appropriateness of recovery groups is required (e.g. do not recover unfamiliar cage-mates within the same cage)
- Clean surgical technique must be practiced, as per LAB_002 Clean Technique for Laboratory Animal Surgery
- Wherever practicable, aseptic surgical technique should be practiced, as per LAB_001 Aseptic Technique for Laboratory Animal Surgery
- Read both LAB_001 and LAB_002 thoroughly and identify which SOP you will use and if any variations are required. This will need to be specified in your AEC approved protocol.
- In the event of equipment failure, or anaesthetic recovery mid-surgery, “alleviating unanticipated pain and distress must take precedence over an individual animal reaching the planned endpoint of the project, or the continuation or completion of the project. If necessary, animals must be humanely killed without delay” (Clause 2.4.18, Australian code for the care and use of animals for scientific purposes 8th Edn., 2013)
- Best practice would include providing oxygen throughout anaesthesia, having at least two people involved in performing anaesthesia and surgery and using aseptic technique. All cannulas and screws should be sterile.

## III. SAFETY AND COMPLIANCE

1. The person undertaking this task must ensure all relevant approvals are in place, and risk assessments have been performed. If unsure, consult your supervisor.
2. Facility protocols should be followed.
3. Possible risks include mouse bite injury, needle stick injury, spills, exposure to infectious agents, repetitive task musculoskeletal injury and psychosocial harm.
4. This procedure must be undertaken by a competent person who has undertaken appropriate training. Training records should be kept. Inexperienced operators should perform this under supervision until deemed competent.

## IV. EQUIPMENT

- PPE*

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see “AEC Reviewed/Approved” date in this document’s header).

*Minimum PPE is gloves, gown and mask, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.*

- Appropriate trolley for transporting cages.
- Disinfectants: surface disinfectant (e.g. 70% ethanol) and skin disinfectants (e.g. chlorhexidine based). Refer to LAB_001 Aseptic Technique for Laboratory Animal Surgery and LAB_002 Clean Technique for Laboratory Animal Surgery for disinfectant options.
- Clean recovery boxes – standard housing boxes including sterile feed, water, appropriate nesting materials (to aid thermal support) and environmental enrichment.
- Active heating equipment (e.g. fit for purpose heat mats, Bair-hugger device, Aria Ventilated Cabinets®)
- Anaesthetic agents – as per AEC approved protocol
- Analgesic agents – as per AEC approved protocol
- Experimental compound – as per AEC approved protocol
- Ophthalmic lubricant (non-medicated, viscous and pH neutral: e.g. Refresh "Lacri-lube"©, Visco-tears© gel)
- Electric clippers
- Stereotaxic device and any required attachments
- Micro drill and supply of fresh drill bits (0.5mm and 0.9mm burr.)
- Surgical instruments: small scissors, sharp straight forceps (Dumont #5), blunt 45° forceps with grip
- Paper towels, benchcoat, betadine, cotton tips, surgical eye spears
- Good quality permanent marker or fine liner, non-toxic, ultra fine point (to mark the skull)
- 1mL syringes, 27g needles, sterile artificial cerebrospinal fluid (ACSF), and/or sterile saline (0.9% NaCl)
- Cannulas, cannula wire dummies, jeweller's screws
- Dental cement (acrylic powder and liquid monomer), small weigh boats, transfer pipette
- Surgery and monitoring record sheets

## V. PROCEDURE

1. Clean and disinfect the workstation using disinfectant (e.g. 70% ethanol) and ensure all equipment is organised of use
2. Prepare clean, warm recovery boxes (e.g. resting on a heat mat).
3. Anaesthetise the animal, as per AEC approved protocol.
4. Apply ophthalmic lubricant to both eyes, using a sterile cotton tip.
5. Using electric clippers, clip fur as required from the head and discard this debris from the workstation.
6. Clip toenails to reduce the chance of scratching post-recovery impacting wound healing

*Steps 3 to 6 should be performed in a separate area to the surgical procedures.*

7. Gently place the mouse/rat into the stereotactic frame using the ear bars and mouth/nose to ensure appropriate positioning (ears first). Local anaesthetic gel should be applied to the skin at the point of contact with the ear bars if they are expected to cause any significant contact pressure.

*Wherever possible the stage of the stereotactic device should have a small heat mat applied (on which the anaesthetised animal rests). If this is not possible given the frame's design, steps 6 to 9 may vary slightly to minimise the time not exposed to active heating – and potential for hypothermia.*

8. If required, position operating light and microscope over the surgical site (i.e. the stereotactic frame).
9. Using the skin disinfectant, clean and disinfect the clipped area of skin over the rodent's skull (i.e. the surgical site), applied using a sterile cotton tip.
10. Check for the absence of a withdrawal reflex. If a withdrawal reflex is present, the animal is not sufficiently anaesthetised and anaesthetic depth needs to be increased prior to proceeding.

*If movement of skeletal muscle, or withdrawal reflexes are present at any point throughout the procedure, activity must stop and only resume once sufficient anaesthetic depth regained. If you are having difficulty*

*maintaining appropriate anaesthetic depth consult a UQBR veterinarian (once the animal has recovered, and before proceeding to anesthetise any more animals).*

**Rodent Preparation for brain and ICV placement**

11. Grip the loose skin over the centre of the scalp with a pair of blunt tissue forceps. Use sharp scissors to cut the flap of skin off. Check that the incision exposes bregma and most of the skull plates between bregma and lambda. If cannulating PFC, it should also expose the target region forward from bregma. (Do not use a scalpel to open the incision in a rodent as you risk cutting the superior sagittal sinus and causing a haemorrhage.)
12. Carefully enlarge the incision if needed – usually cutting in a straight line is easier than trying to remove more skin, with less risk of leaving a jagged or overly large incision.
13. Scrub away the periosteum using a cotton tip wet with 70% ethanol. Dry the skull with another cotton tip.
14. Mark the positions of bregma and lambda using a non-toxic fine point marker. (Only use a pen on the skull when it is dry, or the ink will spread.)
15. Check that the skull is flat. First move to bregma and lower the tip of the cannula so it just touches the skull. Note the DV coordinate. Raise the cannula, move to lambda, and check the DV coordinate here. If the coordinates are the same (or within 0.1mm of each other) the skull is flat. If it is not flat, adjust the height of the jaw bar holder and check again. ALWAYS loosen the nose clamp before adjusting the jaw bar height either up or down. Re-tighten in the new position.

**Supporting screws**

16. Load the first supporting screw onto the screwdriver: hold the screw firmly with forceps. Rest the tips of the screwdriver in the groove on top of the screw, then push the sliding bar downwards so the tips cross over each other and hold the screw. It should be barely tight enough to hold the screw – DO NOT overtighten.
17. Drill holes in the skull for 2 supporting screws. Screw holes should be placed in the rear 1/3 of the parietal bone and laterally towards the edges of the incision but final placement depends on where the cannulas will be fitted. Use a 0.9mm drill bit, and first drill through the full thickness of the skull. Then gently push the drill bit against the edges of the hole to gently enlarge it, very slightly. Ensure you do not make the hole too large for the screw.

*Drilling directly full thickness through the bone (without leaving a thin layer for manual removal) carries the potential to cause unintentional injury to the surface of the brain parenchyma. Optional: stop drilling when only a very thin layer of bone remains and remove manually. The dura should always remain intact and should not be perforated by the drill tip.*

18. Wipe away any blood or bone dust using cotton tips moistened with sterile saline.
19. Insert the supporting screws to about half of their depth (so that the screw does not protrude below the bottom surface of the skull and press on the brain).

**Cannula placement**

20. Move the cannula into place at the target coordinates and ensure it is appropriately secured within the stereotactic apparatus. Lower down until it just touches the skull and mark this place using the marker pen. Then move the cannula out of the way.
21. Using the smallest appropriately sized burr (usually 0.5mm diameter) drill into the cranium.

*If you are cannulating PFC, you will have to pass the cannula through the location of the superior sagittal sinus. Be prepared for substantial bleeding, the best way to stop it is by applying gentle pressure with a cotton bud moistened with sterile saline. If there is a large clot, flush the area with sterile saline and dry with a cotton tip.*

22. Bring the moving arm back into position and return to bregma. Note the new AP coordinate, this will likely be slightly different to the original value.
23. Return to the injection site and slowly lower the cannula to the target position.

*Be prepared to mop up blood that might well around the cannula, depending on position.*

24. Thoroughly dry the surface of the skull using cotton buds, eye spears or both.

25. Repeat steps 19 – 23 if a second cannula is required.

**Finish**

26. Prepare the dental cement, ensuring that you produce a slurry with the consistency of honey. Mix using a clean pipette tip.
27. Once the consistency is correct, drip the dental cement onto the skull building it up around the cannula and supporting screws.

*Don't let the dental cement get on the skin because it will irritate the rodent and cause scratching.*

28. While it sets, give the rodent sterile saline (or equivalent) via subcutaneous injection for rehydration and any necessary analgesia – as per the AEC approved protocol.
29. When the cement has set hard, crack away any cement connecting the cannula holder to the cannula. Loosen the screw holding the cannula onto the holder, and slowly raise the cannula holder until it is well clear of the cannula. Swing the moving arm out of the way.
30. Release the rodent from the ear bars and jaw bar.
31. If needed, use extra dental cement to build up the cap at the front of the head, or to cover up any rough spots.
32. Check that the eyes are clean and reapply eye gel.
33. Put the rodent into the clean cage, belly down, with their chest and head resting on a clean tissue. Put softened pellets (1-2) and gel (spoonful) in the corner of the cage.
34. Put the cage in the recovery chamber. Monitor the animal closely during recovery and do not recover with other animals.
35. When the rodent is sitting in a normal position (regained righting reflex), is able to walk around, and moves normally in response to gentle touch, put dry food pellets and water bottle into the cage and put it in the animal room.
36. Ensure that the surgical monitoring record is complete and fill out a procedure card for the cage.
37. Monitor the animal according to the AEC approved score sheet and protocol.

## VI. BIBLIOGRAPHY

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