

## LAB\_016 Stereotaxic Brain Injections in Mice and Rats

### I. OBJECTIVE

To effect safe and humane injections into the brain of mice and rats via surgery, with the use of a precision stereotactic device.

### II. COMMENTS / RECOMMENDATIONS

- Users must keep monitoring records, which includes surgical records 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.
- PPE is facility dependent, however, this should at least include disposable gloves, long sleeved lab gown, face mask, safety glasses, hair bonnet, closed in shoes.
- 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 recovery 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](#)
- 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 8<sup>th</sup> 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 drill bits should be sterile.

### III. SAFETY AND COMPLIANCE

- The person undertaking this task must ensure all relevant approvals are in place, and risk assessments have been performed. If unsure, consult your supervisor.
- Facility protocols should be followed.
- Possible risks include mouse bite injury, needle stick injury, spills, exposure to infectious agents, repetitive task musculoskeletal injury and psychosocial harm.
- 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.

### III. EQUIPMENT

- 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

#### Conditions:

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- 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
- Local anaesthetic gel (e.g. Emla cream®) <optional>
- Stereotactic device and any required attachments
- Operative light and microscope <optional>
- Sterile surgical instruments and disposables
  - Including: scalpel (suggested: no.11), fine forceps, haemostats, retractors, drill and appropriate drill bits, needle & syringe or micropipette, cotton tips, suture, wound clips or tissue glue/adhesive



A. B.  
Figure. Example set ups for stereotaxic rigs. A) Manual set up and B) computerised stereotaxic rig

#### IV. PROCEDURE

1. Clean and disinfect the workstation using disinfectant (e.g. 70% ethanol) and ensure all equipment is organised of use (including sterile surgical instruments).
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. Check anaesthetic depth by checking 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. Gently place the mouse/rat into the stereotactic frame using the nose/mouth and ear bars to ensure appropriate positioning. 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.

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*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.*

7. If required, position operating light and microscope over the surgical site (i.e. the stereotactic frame).
8. Using the skin disinfectant, clean and disinfect the clipped area of skin over the mouse's skull (i.e. the surgical site), applied using a sterile cotton tip.
9. Again, check anaesthetic depth by checking 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 anaesthetise any more animals).*

10. Using a scalpel perform a small, mid-line, linear incision over the cranium to expose the desired area of injection. Tissue retractors may be useful to keep the skin and associated soft tissue retracted from the surgical site.
11. Relative to anatomical landmarks (e.g. bregma) calibrate the stereotactic apparatus to the desired site of injection, using known coordinates determined by the mouse/rat brain atlases.
12. Ensure the bone drill is appropriately secured within the stereotactic apparatus. Then at the site of injection, using the smallest appropriately sized burr (usually ~1mm diameter) drill directly into the cranium but ensuring the dura remains intact.

*Optional: It is possible to stop drilling when only a very thin layer of bone remains which can then be removed manually. Since drilling 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.*

13. Move the injection syringe or micropipette (loaded with injectate) into place and ensure it is appropriately secured within the stereotactic apparatus.

*Note: stereotactic apparatuses vary in design. Many will require manual detachment, drilling, and then reattachment of the needle. More sophisticated devices will enable more fluid, and in some cases robotic, interchange of needle and drill.*

14. Unless the injection tip expected to easily perforate the dura (which is unusual in all but neonates), use a sterile needle (27G) held at an angle flat to the skull's surface to make an incision in the dura through which you can pass the injection tip. Do not hold the needle perpendicular to the skull when doing this (i.e. downward to the skull) due to the risk associated with accidentally poking, and injuring, the brain's surface with the needle tip.
15. Using the calibrated stereotactic device slowly lower the syringe or micropipette tip through the dura, to the desired depth within the brain tissue. Dependent upon injection parameters, such as depth of the injection within the brain, needle type and experimental compound, the syringe or micropipette tip should remain in place for 1 to 5 minutes, before commencing the injection. This is to permit the brain tissue to conform to the physical changes presented by the insertion of the needle.
16. Inject the experimental compound at a predetermined, constant rate using a syringe pump (50-100nL/minute). Once complete, if the volume injected was >50nL, the needle will need to remain in place for up to 5 minutes to reduce the risk of "back-flow" (retrograde flow along the needle tract).

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17. Once an appropriate period of “rest” has been observed (to prevent back flow), slowly retract the needle, and if required, close the drilled hole with bone wax.  
*Generally, craniotomies <1mm diameter do not require sealing with bone wax.*
18. Using a sterile cotton tip mop up any blood and then close the skin using either skin sutures, wound clips or tissue glue.
19. Administer any approved fluid therapy and/or analgesia
20. Place the animal into a recovery box, maintained on a heat mat until fully ambulatory. If available, recovery boxes may then be placed into a climate controlled, Ventilated Cabinets® for ~12 hour recovery.
21. Clean and disinfect all equipment between each animal.
22. Continuously monitor all mice/rats during surgery and throughout the recovery phase until fully conscious and ambulatory. Mice/rats should be reassessed within 6 hours post recovery, then at least daily for the following 2 days. Ongoing monitoring and analgesia is as described in the AEC approval.

## V. BIBLIOGRAPHY

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