LAB_045 Microinjection of postnatal mice (Expiry: March 2026)

I. OBJECTIVE

To effect safe and humane experimental transfer of compounds into the developing neonatal mouse brain via surgical microinjection into the cerebral ventricle(s), with or without electroporation. Microinjection compounds usually contain experimental plasmid DNA, siRNAs, purified proteins, lipophilic dyes, fluorescent microspheres, inorganic microbeads, drugs, cells, and/or viruses.

II. COMMENTS / RECOMMENDATIONS

- Users must keep monitoring records, which includes surgical records (example templates can be obtained by contacting the UQBR Veterinarians or Animal Ethics Unit Veterinary Officer).
- Any associated experimental compounds or medications 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.
- Clean surgical technique must be practiced, as per LAB_002 Clean Technique for Laboratory Animal Surgery
- Wherever practicable, aseptic surgical technique must be practiced, as per LAB_001 Aseptic Technique for Laboratory Animal Surgery
- Hypothermia is only appropriate as a means of general anaesthesia in neonates up to 7 days of age. **This procedure must not be performed in neonates >7 days of age.**

III. EQUIPMENT

- Disinfectants: surface and skin disinfectants (70% ethanol).
- “Home cage” containing the dam – standard housing boxes including sterile feed, water, appropriate nesting materials (to aid thermal support) and environmental enrichment.
- Temporary holding receptacles (for pups) – dry, soft and heated receptacles (including nesting material from the home cage and an underlying heat mat).
- Anaesthetic equipment – crushed ice +/- water (2-3°C), substrate to avoid pups coming into direct contact with ice (e.g. gauze, nitrile film, or other specific cradle).
- Experimental compounds +/- sterile non-toxic dye for injection (e.g. sterile 1% trypan blue) - as per AEC approved protocol
- Consumables: gauze, cotton tips
- Micro-injection apparatus
  - sterile pulled glass pipette attached to a micropipette
  - alternatively, sterile 30-32G needle attached to a Hamilton syringe
  - Micro-injection tubing may be used to supplement free-hand injection techniques
  - alternatively, stereotactic apparatus
- Microelectrodes
- Electroporation generator
- Fibre optic surgical lamp <optional>

IV. PROCEDURE

1. Prepare yourself and the work station as per LAB_001 Aseptic Technique for Laboratory Animal Surgery / LAB_002 Clean Technique for Laboratory Animal Surgery

2. Prepare the temporary holding receptacles then move half the litter to be injected from the home cage into the first receptacle.

   This procedures describes the use of two receptacles, however, only one may be used at the discretion of the investigator. It is necessary to have some way of identifying injected pups from pups yet to be injected. Often micro-injection compounds contain a grossly observable dye, making the use of one receptacle sufficient.

3. Take one pup from the first temporary holding receptacle and place it onto a bed of crushed ice and water (2-3°C) for 5-8 minutes.

   The pup's skin must not come into direct contact with ice or a highly conductive material (such as a metal) when being chilled. *To avoid this the pup may be cradled within a gauze swab, nitrile film (e.g. from a nitrile glove), paper towel lining a plastic tube or metal tray, or use of some other non-conductive material designed to gently cradle the pup.*

   This method should provide anaesthesia and analgesia for 5 – 15 minutes, and may be prolonged by a further 15 minutes, as required, by maintaining a low body temperature within the pup. This can be achieved through the use of a cold pack (at 3-4°C) once the pup has been removed from the crushed ice bed.

4. Using a cotton tip, moist with 70% ethanol, gently swab the skull over the site for injection.

5. 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).

6. Identify the site of injection using anatomical landmarks (e.g. for the lateral cerebral ventricle, 2/5th distance from the lambda suture to the eye; or 0.8-1mm lateral to the mid-point of the sagittal suture).

   a. If using a free-hand technique for injection

      It may assist to identify the injection site using a non-toxic marker.

      Either a micropipette or Hamilton syringe may be used with or without extension via Micro-injection tubing after it has been appropriately primed.

      Resistance should not be felt when injecting if the needle is correctly positioned within the ventricle. Additionally, if the needle is correctly positioned within the ventricle a characteristic diffusion pattern will be grossly appreciable.

      A maximum of 2uL can be injected.

   b. If using a sterotactic apparatus for injection

      A Hamilton syringe must be used, or device specific micro-injector.

      The injection stage should be chilled and maintained at 0-8°C. If the injection stage has a reservoir, chilling can be achieved with the use of 100% ethanol and dry ice (within the stage reservoir).

      Ensure the head is level prior to injection.

      Following injection, the needle should remain in place for 30-60 seconds, then slowly removed over 1-2 minutes.

      A maximum of 2uL can be injected.

A fibre optic lamp may be used to help visualise the injection site. Iridescent lamps should never be used due to the potential to inappropriately warm cryo-anaesthetised neonates.

7. If electroporation is required to incorporate charged substances such as DNA into a specific tissue region, microelectrodes are placed over the tissue region of interest and 10-80V square wave 50ms pulses (depending on the developmental stage) are administered 3-10 times using an electroporation generator.

   Model specific consideration for electrode placement is required given variable effects associated with differences in direction of charge transfer.

8. Post procedure, move the pup to the appropriate temporary holding receptacle for it to regain normal body temperature, normal skin colour and movement over approximately 5 minutes.

   Continuously monitor all pups during surgery and throughout the recovery phase until fully ambulatory.

9. Once injected make efforts to ensure the pups are marked with the scent of the mother and home cage prior to reunion to reduce the risk of the mother rejecting the pups.

   This is particularly important with fostered pups. Marking can be achieved through the use of home cage nesting material and bedding, among other techniques.

10. Move the remaining un-injected half of the litter from within the home cage to the second (vacant) temporary holding receptacle. At the same time, return the injected pups to the home cage from the other temporary holding receptacle.

11. Repeat the processes described above for the remaining un-injected litter mates.

12. Ensure that the mother is attending to and nursing injected pups within 10 minutes of returning them to the home cage. If the mother is not attending to the pups within this time, or if cannibalism is observed intervention is required.

    Returned pups must be continuously monitored for 10 minutes. If complications are observed during this reunion, communicate plans for intervention with your research group and animal facility staff.

13. Clean and disinfect all equipment between each animal.

V. BIBLIOGRAPHY

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