MOUSE INTRACEREBRAL CANNULATION

ALZET SURGICAL INSTRUCTION SHEET

GENERAL ITEMS

- ALZET Osmotic Pumps*
- ALZET Brain Infusion Kit*
- Loctite 454 Cyanoacrylate adhesive (item #0008670)*
- Homeothermic pad
- Sterile drape
- Sterile gauze, cotton swabs
- Hair clippers (#40 blade)
- 70 % ethanol
- Betadine scrub
- Glass bead sterilizer
- Ocular lubricant

SURGICAL TOOLS

- 7 mm Reflex Wound Clips*
- Reflex Wound Clip Applier*
- Reflex Wound Clip Remover*
- Cannula Holder 1 or 2*
- Scalpel handle #3
- Scalpel blade #15
- Brown Adson forceps
- Hemostat (straight tip)
- Stereotaxic apparatus

* Available from DURECT Corp. (877-922-5938, alzetcs@durect.com)

Pump Preparation

(refer to the package insert for complete filling instructions)

- Fill the empty ALZET pump with your vehicle or drug solution using a syringe and filling tube according to the procedure listed in the package insert included in your box of pumps.
- Insert the flow moderator into the filled ALZET pump until the cap or flange is flush with the top of the pump.
- Prime the filled pumps in sterile saline at 37°C.
    - Refer to your package insert for the appropriate period of priming. Most pumps require at least an overnight priming period.

Anesthesia

Anesthesia is required for surgical implantation of ALZET pumps.

- Anesthetize the animal using either an inhalable (i.e., Isoflurane) or injectable (i.e., Xylazine® and Ketamine®, or sodium pentobarbital) anesthetic.

The use of inhalation anesthetics, such as Isoflurane, is highly recommended. It supplies supplemental oxygen during periods of respiratory depression and provides for rapid anesthetic recovery.

Surgical Preparation

- Apply ocular lubricant to the eye.
- Shave the area centered over the incision site and wash the scalp.

Surgical Procedure

- Place the head of the animal securely and straight in the stereotaxic apparatus with the tooth bar set at an appropriate level as determined by the same stereotaxic atlas used to establish the cannula coordinates.
- Starting slightly behind the eyes, make a midline, sagittal incision and expose the skull. With the rounded end of a spatula, lightly scrape the exposed skull area and pat it dry. Scraping should remove the periosteal connective tissue adhering to the skull. This enables good adhesion of the cyanoacrylate adhesive, which is later used to secure the cannula. Remove all blood with cotton applicators and stop any remaining bleeding with the low-heat cautery device.
- Identify the bone suture junctions bregma and lambda. With these as reference points, determine and mark the location for cannula placement using the stereotaxic apparatus. Create a hole through the skull at the marked, stereotaxically correct location, being careful not to penetrate the dura. This hole will receive the cannula.
- Clean and completely dry the skull area where the cannula will be placed by scraping with the scalpel blade or cotton applicators. Cauterizing blood vessels will stop the additional bleeding.
- Apply a very thin layer of the cyanoacrylate adhesive to the base of the cannula pedestal.

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alzet® OSMOTIC PUMPS

- Insert the cannula, which is attached by tubing to the ALZET pump, through the dry skull. To facilitate precise placement of the cannula, the plastic tab located on top of the cannula can be attached to the stereotaxic apparatus. Alternatively, this tab can be used to insert the cannula by hand.
- Allow the cyanoacrylate adhesive one minute to dry and set.
- Once the cyanoacrylate adhesive is set, gently loosen and remove the cannula holder. The cannula's external arm should lie parallel to the surface of the skull with the tubing extending caudally.
- Prepare a subcutaneous pocket in the midscapular area of the animal's back to receive the ALZET pump. This pocket is created by blunt dissection. The pocket should be large enough to accommodate the pump and permit some pump movement, but not so large as to allow the pump to slip down onto the flank of the mouse.
- Insert the ALZET pump (attached to the catheter and brain cannula) into the subcutaneous pocket. The pump should be placed with the delivery port pointing toward the cannula site. When the pump is properly placed, the catheter should have a generous amount of length to permit free motion of the mouse's head and neck.
- After the cannula is firmly set in place, the plastic tab is easily removed with a heated scalpel, leaving the low-profile cannula in place.
- Close the scalp incision with wound clips or interrupted sutures.

Post Operative Analgesia / Clinical Monitoring and Management

An analgesic can be given post-operatively as needed. Analgesic treatment should be provided under the direction of the staff Veterinarian.

Mice should be monitored daily until the wound clips or sutures are removed then once to twice weekly until completion of the study. It is especially important to check the mouse's health the morning after surgery. Mice that reopen the incision site will typically do so after the first night. If any adverse effects are seen, then inform the staff Veterinarian immediately for appropriate treatment. Potential adverse effects from this procedure are minimal, but may include the following:

- Anesthetic-related respiratory depression: Adverse anesthetic effects can be minimized by proper dosing of anesthetic agents and careful monitoring of animals during the anesthetic period.
- Infection of the incision site: ALZET Osmotic Pumps and Brain Infusion Kits are provided sterile. Infection can be prevented or minimized if trained surgeons use aseptic surgical techniques and maintain the sterility of products being used. Administration of prophylactic antibiotics may be useful in minimizing the risk of infection, and this should be discussed with the staff Veterinarian.
- Post-operative pain or discomfort as evidenced by: decreased activity, decreased food and water intake, weight loss, vocalizations, rough hair coat, hunched posture.

- Remove wound clips 7-10 days post procedure.

Tips on infusion to the mouse brain using the ALZET Brain Infusion Kits:

- Use the spacers provided with the Brain Infusion Kit, to allow proper depth placement of the cannula for the mouse brain.
- Proper cranial coordinates for cannula implantation are essential. A new mouse brain atlas by Franklin and Paxinos has recently been published[1], while two older atlases have been cited with some frequency[2,3].

1 Franklin BJK, Paxinos G; 1997. The mouse brain in stereotaxic coordinates. Academic Press, San Diego, CA.
2 Sidman RL, Angevine JB, Taber PE; 1971. Atlas of the mouse brain and spinal cord. Harvard University Press, Cambridge, MA.
3 Slotnick BM, Leonard CM; 1975. A stereotaxic atlas of the albino mouse forebrain. Rockville, Maryland; Alcohol, Drug Abuse, and Mental Health Administration.

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