Document Number: ANP049	Title: IMPLANTATION OF ALZET PUMP BRAIN INFUSION KIT IN MICE – USING STEREOTAXIC APPARATUS	Effective Date: June 2018
Section: Animal Research		Supersedes Date: N/A
Subsection: Facility	***CONFIDENTIAL INFORMATION*** MOLECULAR MEDICINE RESEARCH INSTITUTE	Page: 1 of 4

1.0 OBJECTIVE

1.1 The objective of this procedure is to describe the procedure for stereotaxic pump implantation in mouse brain.

2.0 SCOPE

2.1 This procedure applies to mice used for experimentation under which research substances / compounds are delivered into the Central Nervous System.

3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to ensure that the animals are treated in a humane manner, according to the Guide for the Care and Use of Laboratory Animals.

4.0 RESPONSIBILITIES

4.1 It is the responsibility of Manager of Animal Research (Mr. Taufeeq Ahmed) or designated alternate to implement this procedure and revise it when necessary.

4.2 The individuals conducting this procedure should possess appropriate experience and training.

5.0 REFERENCES

5.1 SOP# ANP016, Animal Anesthesia

5.2 SOP# ANP043, Subcutaneous Pump Implantation in Rodents

5.3 Video at JOVE demonstrating ICV Delivery to the Mouse CNS

http://www.alzet.com/research_applications/ICV_JoVE.html

5.4 Preparation of the Brain Infusion Assembly

http://www.alzet.com/products/brain_infusion_kit/how_it_works.html

5.5 https://www.stoeltingco.com/neuroscience/stereotaxic/mouse.html

6.0 SAFETY PRECAUTIONS

6.1 It is the responsibility of all personnel to use good judgment and follow any established/posted practices in the laboratories. Protective clothing (e.g., laboratory coats, coveralls, boots, face masks, aprons, rubber gloves and safety glasses) are provided by the company.

6.2 Clean Lab coat or freshly donned disposable smock, Sterile rubber gloves, face masks, bouffants and safety glasses are worn when performing surgery.

6.3 Disposable scalpel blades, hypodermic needles and syringes are placed in a disposable sharps container located in the procedure room. When full, the container is disposed of safely.

6.4 All injury accidents are promptly reported to the appropriate Supervisor.

7.0 EQUIPMENT AND MATERIALS

ALZET Brain Infusion Kits containing assembly for selected type of pump
Autoclaved surgical instruments (1 pair of forceps, 1 curved hemostat, 1 pair of small sharp scissors, 1 pair of small curved blunted scissors, 1 bone cutter, 1 straight hemostat)
Anesthetic (Ketamine and Xylazine combination or isoflurane)
Animal clippers and blade #40
Nolvasan® or Betadine® surgical scrub, 70% Isopropyl Alcohol, hydrogen peroxide
Gauze Sponges, Sterile cotton swabs, 1 bottle of super glue, antibiiotics, eye lubricant
Syringes (1.0 mL)
Suture materials (3-0 to 5-0 black braided silk or Dexon) and or staples/tissue glue.
Heating Pads
Eye Ointment
Antibiotic ointment
Super glue (cyanoacrylate adhesive such as Loctite 454)
Buperonorphine/or NSAIDs
Surgical light
Magnifying loop or dissecting scope.
Mouse Stereotaxic Apparatus- Stoelting Company

8.0 PROCEDURE

8.1 Pre Surgical Procedure

8.1.1 Weigh the animal and mark the tail before which m ay be done before day of surgical session (other identification methods can also be used such as ear tags, ear punch, tattoos etc)

8.1.2 As per Animal Anesthesia SOP, anesthetize animal under Isoflurane or alternatively by injectable anesthetics like Ketamine Xylazine cocktail Monitor depth of surgical plane before initiating procedure by pinching two extremities in two different body quadrants with a surgical instrument with no resultant respobse. . During the procedure, monitor mucus membrane and extremity color plus respiratory character continuously as indications of adequate cardiopulmonary function. Also note any increase in muscle tension, twitching, tearing which might indicate need for additional anesthetic.

8.1.3 Apply Ophthalmic ointment in both eyes to protect corneas and shave the hair from the top of the shoulder up to in between the eyes.

8.1.4 Place the anesthetized mouse on the stereotax with heating pad and push the nose cone over the nose. Secure the head with the ear bars. Reapply eye ointment in both eyes if needed. Inject Buprenorphine subcutaneously at the rate of 0.05-0.1 mg/Kg

8.1.5 Prepare surgical sites with Nolvasan® or Betadine® surgical scrub alternating with 70% Isopropanol (2-3 times).

8.2 Surgical Implantation of Alzet Osmotic Pump

8.2.1 Using forceps and a small pair of scissors, make an incision in the skin from the base of the neck above the animal's skull to up in between the eyes

8.2.2 Insert blunted scissors with the curve facing up and slide underneath the skin at the base of the neck back towards the left hind limb to form the subcutaneous pocket for the pump., and gently open and close its jaws to create a pocket for the pump.

8.2.3 Wipe the skull clean with sterile cotton swabs, followed by cotton swabs dipped in hydrogen peroxide to enhance bregma.

8.2.4 Now using curved hemostat carefully take out filled pump (which was filled and prepared as per Alzet pump filling and storage instructions). If there is a need of primed pump to start delivery immediately then keep pump in saline overnight at 37 degree centigrade. Hold base of the pump with the forceps and push the flow modulator in the rest of the way with the curved hemostat. Using superglue, attach one or two spacer disks, (which are included in Brain Infusion Assembly kit) to the catheter so that the tip of the catheter reaches the appropriate depth into the mouse brain.

8.2.5 Holding with hemostat, insert pump under the skin at the base of the neck and push it back towards the left hind limb as far as it will go without resistance. The delivery port should be pointing towards cannula site. Be careful to not to let catheter touch anything.

8.2.6 With the curved hemostat, grab the cannula at the groove where the top meets the pedestal. Move the cannula driver into position and secure into place.

8.2.7 Place a single drop of super glue on the base of the cannula. Push the top of the cannula into the driver and position so that tubing is pointed straight back.

8.2.8 Wipe the skull clean with sterile cotton swabs and to clear any overlying tissues

8.2.9 Touch the catheter tip to bregma and zero the coordinates on the Digital display. Raise the catheter and move it to desired coordinates determined from mouse brain atlas.. Hold the skin out of the way with curved hemostat.

8.2.10 Drive the thin metal catheter through the skull until the plastic cannula base is securely pressed against the top of the skull. The metal catheter can be driven through the skull in mice due to the relative thin skull.

8.2.11 With the cannula driver holding the cannula/catheter in place, wait 1-2 minutes for the glue to fully dry

8.2.12 Hold the catheter in place with the curved hemostat while raising the cannula driver. Slowly release the hemostat to ensure that the cannula is properly secured to the skull

8.2.13 With a cotton swab, press down on the top of the cannula. Fit the rat bone cutter into the groove between the top and base of the cannula. Clip off the top of the cannula while still pressing down with the cotton swab (If the cannula does come unglued, quickly re-glue and apply pressure with a cotton swab for an additional 2 minutes)

8.2.14 Using the 5-0 suture thread, suture along the full opening by using simple interrupted suture pattern or running horizontal mattress suture pattern. Alternativley, wound metal staples or tissue glue could also be used to close the incision.

8.2.15 Apply a dab of antibiotic ointment over the head and the neck along the incision. Unscrew the ear bars and loosen the nose cone. Remove mouse from the stereotax and place on a warming pad for recovery. Do not leave the mouse unattended until it is awake and regained sternal recumbence. If not already administered, give analgesics and warmed physiological fluids SQ (0.5-1cc). Do not place back in animal holding area until fully conscious and exhibiting normal exploratory and grooming behaviors

8.2.16 Do not put operated animal with non-operated animals. Mice that have gone surgery can be put together.

8.3 Post-operative Care

8.3.1 Monitor the mouse daily after surgery for the first week for any abnormal signs like hunched posture, ruffle hair coat or lethargy. The incision site should be checked daily for any sings of dehiscence or infection. Monitor body weight once a week and if mouse loses more than 20% of body weight, it is euthanized.

8.3.2 Provide moist food pellets on floor for easy access

8.3.3 If a mouse appears to be in pain and squeaking, inject Buprenorphine subcutaneously. If needed, repeat every 8-12 hours. If mouse is dehydrated, confirmed by tenting of skin, inject 0.5ml to 1ml of saline subcutaneously

8.3.4 Remove the sutures or metal clips 7-10 days following surgery to prevent irritation