1.0 OBJECTIVE

1.1 The objective of this procedure is to describe the procedure for jugular vein cannulation in rats.

2.0 SCOPE

2.1 This procedure applies to animals used for experimentation.

3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to ensure that the health and well being of employees is protected, and that potentially hazardous procedures are performed in a safe manner.

4.0 RESPONSIBILITIES

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

5.0 REFERENCES

5.1 SOP# ANP016, Animal Anesthesia.

6.0 SAFETY PRECAUTIONS

6.1 It is the responsibility of all personnel to use good judgment and safe 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 Sterile rubber gloves, facemasks, bouffants and safety glasses are worn when performing surgery.

6.3 Used 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

Autoclaved surgical instruments
Anesthetic (Domitor® and Ketamine combination or Ketamine and Xylazine combination or isoflurane)
Animal clippers and blade #40
Nolvasan® or Betadine® surgical scrub and 70% Isopropyl Alcohol

Gauze Sponges
Syringes (1.0 mL)
Blunt needles (gauge 22 or 23)
Suture materials (3-0 to 5-0 black braided silk or Dexon) or staples.
Micro-Renathane, Type MRE 040 catheter or equivalent
0.9 % sterile saline
Lactated Ringers Solution
Heating Pads
Heparin (1,000 or 10,000 units/mL)
Glycerol (99.5 % pure)
Catheter Introducer
Surgical light
Magnifying loop or dissecting scope.

8.0 PROCEDURE

8.1 Preparation of Catheter

8.1.1 A polyurethane catheter, with 0.04 mm outer diameter (O.D.)and 0.025 mm inner diameter I.D.)(Micro Renathane, Braintree Scientific, MA) is cut squarely with a scalpel blade into 15 cm lengths.

8.1.1 Measure a length of 2.5 – 3.5 cm from one end of the catheter and mark it with a permanent marker, the length depending on the size of the rats.

8.1.2 Cut off about 2 mm piece from the other end to be used as cuff. Using a hemostat, its diameter is widened so that the catheter can be inserted up to the marked distance.

8.1.3 Soak the tip of the catheter overnight in heparinized saline (100 units/mL).

8.1.4 On the day of the procedure, a blunt 22 G x 1 cm or 23 G x 1 cm needle is attached to the unmarked end of the catheter and connected to a 1 cc syringe filled with saline. The catheter is then filled with saline solution

8.2 Catheterization of the Jugular Vein

8.2.1 Anesthetize the animal using the proper dose of Ketamine and Domitor combination or Ketamine and Xylazine combination or isoflurane. Monitor surgical plane by toe pinch reflex. During the procedure, monitor color and respiration periodically.

8.2.2 Once the animal is anesthetized, shave the ventral neck area (3-4 cm^2) and dorsal neck area (3-4 cm^2).

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

8.2.4 Place rat in dorsal recumbency.

8.2.5 Make a 1-2 cm skin incision on the ventral neck.

8.2.6 Dissect jugular vein free from the surrounding tissues.

8.2.7 Place two ligatures under the vein, one at cranial end and one at caudal end of the vessel.

8.2.8 Tie the cranial ligature (do not cut ends), gently retract with forceps.

8.2.9 Transect the vessel using small spring scissors, between the two ligatures.

8.2.10 Insert catheter introducer into the vessel lumen.

8.2.11 Insert the saline filled cannula via the groove of the introducer. Once inside the vein, the introducer is pulled out and the cannula (attached to an adapter and saline filled syringe) is inserted further towards the heart until the cuff is inside the vein (approximately 25-35 mm depending on the size of the rat).

8.2.12 Withdraw enough blood into the cannula to ensure proper placement; flush with saline. If satisfactory, the blood should be flushed back into the circulation.

8.2.13 Tie caudal ligature around cannula behind the cuff to secure.

8.2.14 Tie cranial ligature around cannula.

8.2.15 Withdraw enough blood to ensure patency; flush with saline.

8.2.16 Cut excess ligature material.

8.2.17 Place the animal in ventral recumbency.

8.2.18 Make 1-2 cm skin incision caudal to the plane of the ears.

8.2.19 Using a hemostat, dissect a subcutaneous passage to the ventral incision and grab the cannula below the tip of the blunt needle.

8.2.20 Remove syringe and adapter from cannula.

8.2.21 Pass the end of the cannula through the subcutaneous passage.

8.2.22 Secure a small loop of cannula subcutaneuosly to the skin near the incision site to anchor the cannula.

8.2.23 Check patency by drawing blood into cannula; flush with saline.

8.2.24 Close the ventral incision with 3-0 to 5-0 suture or staples.

8.2.25 Fill cannula with heparinized glycerol solution or heparinized PVP solution (approximately 50 uL of 500 IU heparin per 1 mL final solution).

8.2.26 Insert the metal plug halfway.

8.2.27 Close the dorsal incision with 3-0 to 5-0 suture or staples.

8.2.28 Administer 3-5 mL Lactated Ringers Solution subcutaneously and keep the animal in a heating pad until recovery. Administer anesthetic reversal agent if available.

8.2.29 Return the animal into individual cage lined with paper towels. Observe until the rat shows a righting reflex. Provide food and water.

8.2.30 Monitor animal and incision site for 3-7 days.

8.3 Catheter Maintenance

8.3.1 Animals should be housed individually.

8.3.2 Replace heparin lock solution every 5 days to help maintain patency.

Note:

Unless euthanasia is required at the end of a study, cannulated rats may be allowed to recover. Recovered animals may be used for other purposes at the discretion of the investigator.