Chronic Sampling and Delivery methods In Mice and Rats

IACUC Standard Procedure
Effective Date: May 2025

UCSF Office of Research
Institutional Animal Care and Use Program

I. Osmotic Pump Implantation (Subcutaneous and Intraperitoneal)

The following policies must be followed for all implantation procedures:

- UCSF IACUC Guidelines for Rodent Anesthesia
- UCSF IACUC Guidelines for Rodent Surgery
- Pumps must be sterile and solutions infused need to be of pharmaceutical grade unless otherwise specified in the protocol.
- IACUC approval is needed if the pump is to be replaced. Also, explanted pumps cannot be re-used.
- When replacing pumps, it may be advisable not to reuse the location of the prior pump, as scar tissue may inhibit intended uptake.
- Consult with a LARC veterinarian if you have questions regarding pump placement or replacement.

The protocol must identify:

- The anesthetics and analgesics to be used. Multimodal analgesia is expected and should be stated in the protocol.
- Size of the pump implanted (determine from the size of the animal and manufacture's guidelines). The pump size should allow the animal to wear it comfortably.
- The location where the osmotic pump will be implanted. If pumps will be used with indwelling cannulae (brain, vascular, etc.), check the applicable Standard Procedure for Indwelling Vascular or Intracranial Cannulae in F.1, in addition to checking off this Standard Procedure for the pump placement aspect of the procedure.
- Solution to be infused (in the Experimental agents section).
- How long the pump will be in place (refer to the manufactures guidelines).
- If the pump will be replaced, provide justification and the number of replacements.
- If more than one osmotic pump/animal will be implanted, this must be described and justified.

Description of Procedure:

A. Subcutaneous Implantation: The common site for subcutaneous implantation of osmotic pumps in mice and rats is on the dorsolateral body wall, slightly caudal to the scapulae. Other regions may be used, provided that the pump does not put pressure on spine, vital organs, or impede respiration or locomotion.

1. Once the animal has been anesthetized and the intended area has been shaved free of hair and aseptically prepared, make an incision 1.5 times the diameter of the shorter length of the pump.
2. Spread the subcutaneous tissue to create a pocket for the pump. The pocket should be large enough to allow some free movement of the pump but not so large that it will slip.
3. Insert a filled pump into the pocket, with the delivery portal side of the pump entering the pocket first. Assure the pump rests past the incision, and does not sit immediately

beneath the incision so as to avoid tension over the incision. It is important for wound edges to close easily.
4. Close the wound with wound clips or suture appropriate to the size of the animal (recommended wound clip size: mice 7 mm; rats 9 mm).
5. Recover the animal the animal following the UCSF Rodent Anesthesia Guidelines and provide analgesia as described in the approved protocol.

B. Intraperitoneal Implantation:

1. After anesthetizing, place mouse in dorsal recumbency.
2. Once the intended area has been shaved free of hair and aseptically prepared, make a 1 cm midline skin incision on the abdomen.
3. Incise the peritoneal wall along the linea alba directly beneath the skin incision.
4. Insert a filled pump, delivery portal first, into the peritoneal cavity along the body wall.
5. Close the musculoperitoneal layer with absorbable suture taking care to avoid damage to the underlying bowel.
6. Close the skin incision with wound clips or suture (recommended wound clip size: mice 7 mm; rats 9 mm).
7. Recover the animal following the UCSF Rodent Anesthesia Guidelines and provide analgesia as described in the approved protocol.

C. Explantation and Replacement of Osmotic Pumps:

1. The initial incision site should not be re-used.
2. A new incision site may be made adjacent to the original, along the short axis of the pump.
3. If the pump has been in place longer than two weeks, and/or the infusate is an irritant, it may be necessary to free the pump from surrounding connective tissue in order to remove it.
4. Close the incision with wound clips or suture.
5. Recover the animal following the UCSF Rodent Anesthesia Guidelines and provide analgesia as described in the approved protocol.

Protocol Requirements:
Surgery: Note 'Survival Surgery.' Also note 'Multiple Survival Surgery' if explanting and replacing pumps.

Agents: This procedure requires anesthetics and analgesics. All agents administered to animals should be listed in the "Agents" section of RIO.

Adverse Effects to be considered: Infection, dehiscence, skin ulceration

Resource Consulted:
https://www.alzet.com/guide-to-use/implantation_and_explanation/

II. Indwelling Vascular Cannula Placement In Mice and Rats

The following policies must be followed for all implantation procedures:

- UCSF IACUC Guidelines for Rodent Anesthesia
- UCSF IACUC Guidelines for Rodent Surgery

Description of Procedure:

A. Vascular cannulae placement may be used for direct drug administration or blood sampling. The following procedure details the placement of a cannula in the external jugular vein. This is a general example of a preferred site because of its size and ease of access. Alternatively, other sites may be used if described in the protocol Section G.

1. Prepare the appropriate size catheter for placement.
2. Anesthetize and prep the animal following the UCSF Rodent Anesthesia Guidelines and Rodent Surgery Guidelines.
3. Position the animal in dorsal recumbency and elevate the neck to display the ventral neck.
4. Make an incision just lateral to the trachea and dissect down to the external jugular vein so that it can be elevated.
5. Ligate the cephalic end of the vein and place two loose ligatures around the cardiac end of the vein.
6. Insert the catheter into the jugular vein and control hemorrhage with gentle traction on the cephalic suture ends.
7. Tie the cardiac ligatures around the catheter and then tie the cephalic ligature. Trim the ends of all three ligatures close to the knots.
8. Use a two-layer closure with absorbable material in the underlying fascia if possible and an additional layer of closure for the skin (using wound clips or suture).
9. Recover the animal following the UCSF Rodent Anesthesia Guidelines and provide analgesia as described in the approved protocol.
10. Describe in Section G. the maximum duration for cannula maintenance and how you will care for the externalized cannula to maintain patency and asepsis.

B. Vascular cannulae may also be connected to an implanted pump.
In the RIO protocol Section F.1, select both “Indwelling Cannula, Vascular” and the applicable osmotic pump implantation (subcutaneous and/or intraperitoneal)
Follow Section I above for osmotic pump implantation.
In the RIO protocol Section G, describe the tunneling method to connect the cannula to the pump.

Protocol Requirements:

Section G. Procedures:

- Describe method if using a site other than external jugular vein for vascular cannula placement.
- Describe care of externalized cannula to maintain patency and asepsis.
- If connecting vascular cannula to implanted pump, describe tunneling method.

Section I. Agents: This procedure requires anesthetics and analgesics. All agents administered to animals should be listed in the “Agents” section of RIO.

Section J. Adverse Effects to be considered: Infection, dehiscence, skin ulceration, occlusion/ loss of patency.

III. Indwelling Intracranial Cannula Placement

Local Administration of Drugs to the CNS: Direct access to the CNS is useful for testing those agents that affect the CNS but do not cross the blood brain barrier appreciably.

Procedure for rats:

1. Anesthetize and prep the animal following the UCSF Rodent Anesthesia Guidelines and Rodent Surgery Guidelines and then place the animal in a stereotaxic apparatus.
2. A local block is recommended before making the skin incision. Make an incision to expose the skull and remove the periosteal connective tissue.
3. Identify bone suture junctions bregma and lamda to determine and mark the location for cannula placement.
4. Drill a hole in the marked, correct stereotaxic location. This hole will receive the cannula.
5. Insert the cannula (attached to the pump if needed) through the skull and cement in place.
6. Drill a second hole partially through the skull lateral to the cannula to attach a small screw to anchor the cannula taking care not to go through the entire cranium. The screw should extend 1-2 mm above the skill.
7. Dry the skull surface and cover the cannula, implantation site and anchor screw with dental cement or cyanoacrylate adhesive.
8. After the cement has set, if you are including a pump, follow Section I above for osmotic pump implantation. In Section F.1 of the RIO protocol, select both the applicable osmotic pump implantation (subcutaneous and/or intraperitoneal) and "Indwelling Cannula, Intracranial." Describe in Section G of the RIO protocol any variations to this method and the tunneling method if connecting cannula to an implanted pump.
9. Describe care of externalized cannula to maintain patency and asepsis.
10. Close any incisions with wound clips or suture. Recover the animal following the UCSF Rodent Anesthesia guidelines and provide analgesia as described in the approved protocol.

For mice: Follow the procedure as detailed for rats with the following exceptions:

- Do not use a stay screw or dental cement as the skull is too thin to support a stay screw.
- The upper portion of the plastic cannula which is used for attachment to the stereotax arm needs to be removed prior to closing the incision.

Protocol Requirements:

Section G. Procedures:

- Describe care of externalized cannula to maintain patency and asepsis.
- If connecting vascular cannula to implanted pump, describe tunneling method.

Section I. Agents: This procedure requires anesthetics and analgesics. All agents administered to animals should be listed in the "Agents" section of RIO.

Section J. Adverse Effects to be considered: Infection, dehiscence, skin ulceration, neurological deficits.