
1. PURPOSE

The intent of this Standard Operating Procedure (SOP) is to describe procedures for rodent stereotaxic surgery.

2. RESPONSIBILITY

Principal investigators (PI) and their staff, veterinary care staff or any individual performing stereotaxic surgery on rodents, or assisting in those procedures.

3. MATERIALS

- 3.1. Anesthetics
- 3.2. Non-steroidal analgesic (such as carprofen or meloxicam; see Rodent Analgesia SOP)
- 3.3. Lidocaine-bupivacaine, 1:1 mixture (local analgesic)
- 3.4. Sterile 0.9% saline
- 3.5. Sterile ophthalmic ointment
- 3.6. Electric razor
- 3.7. Gauze
- 3.8. Antiseptic detergent (e.g., chlorhexidine 2% solution or povidone-iodine solution)
- 3.9. 70% alcohol
- 3.10. Heating disc, warming pad or warm-water circulating pad (do not use electric heating pads), insulating material (thermal drapes, bubble wrap)
- 3.11. Sterile surgical instruments
- 3.12. Sterile gauze and swabs
- 3.13. Sterile drapes
- 3.14. Drill, sterile stainless steel screws, sterile cannulae
- 3.15. Suture material or wound clips (Autoclips)
- 3.16. Dry bead sterilizer or cold sterilization agents (e.g. glutaraldehyde) and 70% alcohol (as a rinsing agent)

4. PROCEDURES

- 4.1. Document the details of the surgical procedure in the Rodent Procedure Log.
- 4.2. Pre-operative Care:
 - 4.2.1. Perform pre-operative procedures at a safe distance from the surgical environment in order to prevent contamination with hair.
 - 4.2.2. Anesthetize the animal according to Rodent Anesthesia SOP (can be done after step 4.2.3).
 - 4.2.3. Administer a non-steroidal anti-inflammatory analgesic, e.g., carprofen or meloxicam, as per rodent analgesia SOP.
 - 4.2.4. Administer from 0.2 to 0.5mL/10g body weight of 0.9% sterile saline subcutaneously.
 - 4.2.5. Shave top of the head and remove hair.
 - 4.2.6. Secure animal in the stereotaxic frame.
 - 4.2.7. Apply ophthalmic ointment in both eyes to prevent corneal desiccation. Reapply as needed.
 - 4.2.8. Place a heat source under the animal or wrap the animal with insulating material, e.g., thermal drapes, bubble wrap.

- 4.2.1. Apply 70% alcohol with gauze or swabs to the surgical site. Be careful not to wet a large area on the animal as the evaporation of alcohol will lead to heat loss.
- 4.2.2. Wash the surgical site with 2% chlorhexidine solution or povidone-iodine solution.
- 4.2.3. Repeat steps 4.2.8 and 4.2.9 twice.
- 4.2.4. Surgeon's preparation:
 - 4.2.4.1. Wash hands.
 - 4.2.4.2. Wear a surgical mask and a clean gown.
 - 4.2.4.3. Use aseptic technique.
 - 4.2.4.4. Wear sterile or alcohol disinfected gloves.
 - 4.2.4.5. The surgeon must avoid touching non-sterile surfaces.
- 4.3. Stereotaxic surgery:
 - 4.3.1. Ensure that all the available materials are at hand.
 - 4.3.2. Begin surgery with clean and sterile surgical instruments, handle them aseptically.
 - 4.3.3. Designate a sterile area on the working surface for the sterile material (instruments, suture material, gauze, etc.).
 - 4.3.4. Prior to surgery, verify depth of anesthesia by loss of animal's pedal withdrawal (toe pinch) reflex.
 - 4.3.5. Cover the animal with a sterile, opaque, drape. Surgical drapes must be sterile for the first animal, and may then be transferred to the following animal during serial surgeries. The top surface of the drape must never come in contact with non-aseptic areas, and must not be soiled.
 - 4.3.6. Use the drape to shield the animal's eyes from surgical lights as prolonged exposure to intense light may cause damage to the retina.
 - 4.3.7. Expose the cranium by making a surgical anterior-posterior incision with a scalpel blade (for lesions) or by cutting a circular fold of skin with scissors (for cannula placement).
 - 4.3.8. Avoid contact of tissues with fingers by using the tip of instruments.
 - 4.3.9. Reflect the skin.
 - 4.3.10. Apply local anesthetics (mixture of bupivacaine and lidocaine) on the periosteum.
 - 4.3.11. Scrape the skull and wipe the skull surface with sterile swabs to remove blood.
 - 4.3.12. Using a hand-held drill, drill burr holes (up to 2mm in diameter for rats and 1mm in diameter for mice) in to the skull for the insertion of stainless steel securing screws.
 - 4.3.13. Insert stainless steel screws.
 - 4.3.14. Make a craniotomy over the target area of the brain.
 - 4.3.15. Guide cannulae, temporarily fitted with internal cannulae, are inserted into the target area according to appropriate stereotaxic coordinates.
 - 4.3.16. Dental acrylic is applied around the cannulae, screws and any exposed cranium to secure the cannulae in place.
 - 4.3.17. When the cement hardens, the internal cannulae are replaced with "dummy" cannulae (obdurators) which are inserted in the guide cannulae to maintain patency.
 - 4.3.18. A plastic dust cap is placed to protect the cannulae assembly.
 - 4.3.19. Infiltrate the wound with a local anesthetic, e.g., mixture of lidocaine and bupivacaine, prior to closing the skin. Refer to Rodent Analgesia SOP.
 - 4.3.20. Skin is sutured with polyamide-nylon, PDS, Vicryl; size: 3-0 or 5-0 or incision can be closed using wound clips (Autoclips); 7mm or 9mm. Sutures or staples must be removed after 10 days.
 - 4.3.21. Disinfect the instruments between each animal by dipping them in a hot glass bead sterilizer for approximately 30 seconds after removing any blood or debris (let cool completely) or in liquid sterilizing solution (e.g., glutaraldehyde or equivalent) for >5 minutes and then rinse with 70% alcohol. For liquid sterilization, it is recommended to use two alternating surgical kits in order to increase contact time with the solution.

4.3.22. Dip suture material in 70% alcohol between each animal.

4.4. Surgical Monitoring and Supportive Care:

4.5. Refer to Rodent Surgery SOP. Post-operative Care:

4.5.1. Refer to Rodent Surgery SOP.

4.5.2. Repeat the administration of a non-steroidal anti-inflammatory analgesic (such as carprofen or meloxicam as per rodent analgesia SOP), every 24 hours for 1 to 2 days.

5. REFERENCES

- 5.1. Gardiner TW, Toth LA. Stereotactic Surgery and Long-Term Maintenance of Cranial Implants in Research Animals. *Contemp Top Lab Anim Sci.* 1999 Jan;38(1):56-63.
- 5.2. Bradfield JF, Schachtman TR, McLaughlin RM, Steffen EK. Behavioral and physiologic effects of inapparent wound infection in rats. *Lab Anim Sci.* (1992). Dec;42(6):572-8.
- 5.3. Noell WK, Walker VS, Kang BS, Berman S. (1966). Retinal damage by light in rats. *Invest Ophthalmol Vis Sci* 5:450-473
- 5.4. J. Lanum, The damaging effects of light on the retina. Empirical findings, theoretical and practical implications. *Surv Ophthalmol.* (1978). Jan-Feb; 22(4): 221–249.

SOP REVISION HISTORY

DATE	PREVIOUS VERSION	NEW VERSION
2018.03.21	(NO TEXT)	3.13 Sterile drapes
2018.03.21	(NO TEXT)	4.1. Document the details of the surgical procedure in the Rodent Procedure Log.
2018.03.21	4.2.8 Wash the surgical site with 2% chlorhexidine solution or povidone-iodine solution. Be careful not to wet the animal. 4.2.9. Apply 70% alcohol with gauze or swabs to the head. Be careful not to wet a large area on the animal as the evaporation of alcohol will lead to heat loss.	4.2.1. Apply 70% alcohol with gauze or swabs to the surgical site. Be careful not to wet a large area on the animal as the evaporation of alcohol will lead to heat loss. 4.2.2. Wash the surgical site with 2% chlorhexidine solution or povidone-iodine solution.
2018.03.21	(NO TEXT)	4.3.6. Use the drape to shield the animal's eyes from surgical lights as prolonged exposure to intense light may cause damage to the retina.
2018.03.21	4.3.7. Apply local anesthetics (mixture of bupivacaine and lidocaine) on the periosteum. 4.3.8. Reflect the skin, scrape the skull and wipe the skull surface with sterile swabs to remove blood.	4.3.8 Reflect the skin. 4.3.9 Apply local anesthetics (mixture of bupivacaine and lidocaine) on the periosteum. 4.3.10 Scrape the skull and wipe the skull surface with sterile swabs to remove blood.
2018.03.21	(NO TEXT)	References in section 5.

Investigator:	Protocol:
Procedure: Stereotaxic Surgery	Performed by:

Instructions: complete this log for rodent procedures requiring anesthesia, analgesia or post-procedure care (ex. surgeries, experimental infection). Keep the log in the housing room while active and in your files for 3 years for future review by the Quality Assistant and/or the FACC.

ANALGESIA

- carprofen: mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- buprenorphine: mouse: 0.1mg/kg SC or IP every 4-8 hrs;
rat: 0.05mg/kg, SC or IP, every 8-12 hrs
- lidocaine/bupivacaine (local analgesic)
- other: _____

ANESTHESIA

- isoflurane 2-2.5%
- ketamine/xylazine/acepromazine*:
mouse: 100 mg/kg (K)- 10 mg/kg (X)- 3 mg/kg (A) IP
rat: 50 mg/kg (K)- 5 mg/kg (X)- 1 mg/kg (A); IP or IM
- other: _____

OTHER AGENTS ADMINISTERED

- _____
- _____
- _____

Animal ID	Date	Anesthesia		Analgesia		Other		Heat Source Provided		Recovery time	Comments/observations	Initials
		dose	time	dose	time	dose	time	procedure	recovery			
1								<input type="checkbox"/>	<input type="checkbox"/>			
2								<input type="checkbox"/>	<input type="checkbox"/>			
3								<input type="checkbox"/>	<input type="checkbox"/>			
4								<input type="checkbox"/>	<input type="checkbox"/>			
5								<input type="checkbox"/>	<input type="checkbox"/>			
6								<input type="checkbox"/>	<input type="checkbox"/>			
7								<input type="checkbox"/>	<input type="checkbox"/>			
8								<input type="checkbox"/>	<input type="checkbox"/>			
9								<input type="checkbox"/>	<input type="checkbox"/>			
10								<input type="checkbox"/>	<input type="checkbox"/>			
11								<input type="checkbox"/>	<input type="checkbox"/>			
12								<input type="checkbox"/>	<input type="checkbox"/>			
13								<input type="checkbox"/>	<input type="checkbox"/>			
14								<input type="checkbox"/>	<input type="checkbox"/>			

Comments/footnotes:

*Dose can vary with the sex, the age, the strain, and the body condition of the animal.

ANALGESIA

- carprofen: mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- buprenorphine: mouse: 0.1mg/kg SC or IP every 4-8 hrs; rat: 0.05mg/kg, SC or IP, every 8-12 hrs
- OTHER _____

Initial the appropriate boxes when completed

	Animal ID	Date	Analgesia			SC fluids			Wet food			Time			Remove Sutures (Day 7-10)
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	
1															
2															
3															
4															
5															
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7															
8															
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10															
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14															
Comments/footnotes:															