
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 clipper
- 3.7. Gauze
- 3.8. Antiseptic solution for skin (e.g., chlorhexidine 2% solution or povidone-iodine solution, 70% alcohol, or 2% chlorhexidine in 70% alcohol solution)
- 3.9. 3% Hydrogen peroxide
- 3.10. Heating disc, warming pad or warm-water circulating pad (do not use electric heating pads unless specifically designed for use with laboratory rodents), 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, sterile Hamilton syringe
- 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. Administer a non-steroidal anti-inflammatory analgesic, e.g., carprofen or meloxicam, as per rodent analgesia SOP.
 - 4.2.3. Anesthetize the animal according to Rodent Anesthesia SOP (can be done after step 4.2.3).
 - 4.2.4. Apply ophthalmic ointment in both eyes to prevent corneal desiccation. Reapply as needed.
 - 4.2.5. Administer from 0.2 to 0.5mL/10g body weight of 0.9% sterile saline subcutaneously.
 - 4.2.6. Shave top of the head and remove hair.
 - 4.2.7. Wash the surgical site with 2% chlorhexidine solution or povidone-iodine solution. Be careful not to wet the animal.
 - 4.2.8. Secure animal in the stereotaxic frame.

- 4.2.9. Place a heat source under the animal or wrap the animal with insulating material, e.g., thermal drapes, bubble wrap.
- 4.2.10. Preparation of the surgical site:
 - 4.2.10.1. When using a 2% chlorhexidine in 70% alcohol solution, apply 3 times with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Be careful not to wet a large area on the animal as the evaporation of the solution will lead to heat loss.
 - 4.2.10.2. If using 2% chlorhexidine solution or povidone-iodine solution, first apply 70% alcohol with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Be careful not to wet a large area on the animal as the evaporation of alcohol will lead to heat loss. Then apply 2% chlorhexidine solution or povidone-iodine solution with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Repeat these steps two more times.
- 4.2.11. Surgeon's preparation:
 - 4.2.11.1. Wash hands.
 - 4.2.11.2. Wear a surgical mask and a clean gown.
 - 4.2.11.3. Use aseptic technique.
 - 4.2.11.4. Wear sterile or alcohol disinfected gloves.
 - 4.2.11.5. 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 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 drape.
 - 4.3.5.1. The drape can be placed only when suturing the wound to prevent sutures from coming into contact with hair and skin around the surgical area.
 - 4.3.5.2. 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.
 - 4.3.5.3. Glad® Press'n Seal® wrap can be used as a surgical drape to cover the animal. As it is transparent, it allows for easier monitoring of the animal.
 - 4.3.5.4. Use the drape to shield the animal's eyes from surgical lights as prolonged exposure to intense light may cause damage to the retina.
 - 4.3.6. Expose the cranium by making a surgical anterior-posterior incision with a scalpel blade (for lesions or injections) or by cutting a circular fold of skin with scissors (for cannula placement).
 - 4.3.7. Avoid contact of tissues with fingers by using the tip of instruments.
 - 4.3.8. Reflect the skin.
 - 4.3.9. Apply local anesthetics (mixture of bupivacaine and lidocaine) to the periosteum.
 - 4.3.10. Scrape the skull to detach and push aside the periosteum. Wipe the skull surface with sterile swabs to remove blood.
 - 4.3.11. Hydrogen peroxide solution can be applied to the skull to facilitate identification of bregma; the sutures will appear white.
 - 4.3.12. Intracranial injection:
 - 4.3.12.1. Using predefined stereotaxic coordinates, mark on the skull the intended site of injection.
 - 4.3.12.2. Using a hand-held drill, make a single burr-hole in the skull at the injection site.

- 4.3.12.3. Position the syringe over the burr-hole.
 - 4.3.12.4. Lower the syringe until the needle touches the cortical surface and use this point as “zero” (Z zero). Lower the syringe needle to the desired depth.
 - 4.3.12.5. Inject slowly to avoid an acute increase of intracranial pressure and facilitate diffusion of the fluid. Depending on the total volume injected, this step may take up to 10 minutes.
 - 4.3.12.6. After completing the injection, you may allow 2-5 additional minutes rest time before starting to withdraw the syringe.
 - 4.3.12.7. Withdraw the syringe slowly.
 - 4.3.12.8. It is not necessary to seal the burr-hole.
 - 4.3.12.9. Infiltrate the wound with a local anesthetic, e.g., mixture of lidocaine and bupivacaine, prior to closing the skin. Refer to Rodent Analgesia SOP.
 - 4.3.12.10. 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.
- 4.3.13. Implantation of cannulae:
- 4.3.13.1. Using a hand-held microdrill, 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.
 - 4.3.13.2. Insert stainless steel screws.
 - 4.3.13.3. Make a craniotomy over the target area of the brain.
 - 4.3.13.4. Guide cannulae, temporarily fitted with internal cannulae, are inserted into the target area according to appropriate stereotaxic coordinates.
 - 4.3.13.5. Dental acrylic is applied around the cannulae, screws and any exposed cranium to secure the cannulae in place. The dental acrylic should create a strong head cap fixed to the skull. The edges of the head cap should be smooth and should cover all of the bone.
 - 4.3.13.6. When the cement hardens, the internal cannulae are replaced with “dummy” cannulae (obdurators) which are inserted in the guide cannulae to maintain patency.
 - 4.3.13.7. A plastic dust cap is placed to protect the cannulae assembly.
- 4.3.14. Implantation of neural implants:
- 4.3.14.1. 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.
 - 4.3.14.2. Insert stainless steel screws.
 - 4.3.14.3. Make a craniotomy over the target area of the brain according to the predetermined stereotaxic coordinates.
 - 4.3.14.4. Slowly and carefully insert implant(s) into the target area(s)
 - 4.3.14.5. Apply dental acrylic around the electrodes, screws and any exposed cranium to secure in place. The dental acrylic should create a strong head cap fixed to the skull. The edges of the head cap should be smooth and should cover all of the bone.
- 4.3.15. 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).
- 4.3.16. Dip suture material in 70% alcohol between each animal.
- 4.3.17. Recommended suture and wound closure:

TISSUE	SUTURE MATERIAL	SIZE	NEEDLE
Skin*	PDS Vicryl Polyamide-nylon	3-0, 4-0, 5-0, 6-0	Cutting Reverse-cutting
	Wound clips (Autoclips)	7mm or 9mm	

* Silk suture should be avoided for skin closure as it may cause a local inflammatory tissue reaction and is associated with a higher incidence of wound infection.

- 4.4. Surgical Monitoring and Supportive Care:
 - 4.4.1. Provide a contact heat source to prevent hypothermia.
 - 4.4.2. Frequently monitor the presence of reflexes, the respiratory rate and breathing pattern, and when available, the heart rate.
 - 4.4.3. Adjust the depth of anesthesia according to monitored parameters (presence of reflexes, respiratory rate and breathing pattern, heart rate).
 - 4.4.4. In the case of respiratory arrest, stop anesthesia, administer oxygen and compress the thorax rapidly rapidly between the thumb and index at a frequency of 80-120/min.
- 4.5. Post-operative Care:
 - 4.5.1. Post-operative care begins immediately following surgery, lasts a minimum of 3 days, and extends for up to 10 days.
 - 4.5.2. Post-operative animals should be identified with a Post-Procedure cage card.
 - 4.5.3. Do not return animals that have not completely recovered to an animal housing room.
 - 4.5.4. Observe the animal until it regains righting reflexes; do not leave recovering animal unattended. Observe respiration and coloration of the eyes (for albinos), mucous membranes, and skin.
 - 4.5.5. Prevent heat loss and maintain the animal in contact with a heat source or inside a heated cabinet until it regains righting reflexes.
 - 4.5.6. Administer oxygen if necessary.
 - 4.5.7. For surgeries exceeding 60 minutes, or if there has been significant blood loss, administer an additional 0.2 to 0.5mL/10 g body weight of isotonic fluids, subcutaneously.
 - 4.5.8. Monitor animals daily for at least the first 3 days following the surgery. Continue daily monitoring and contact veterinary care staff if recovery is prolonged beyond 3 days. Record supportive care provided on the Post-Procedure cage card.
 - 4.5.8.1. Repeat analgesics post-surgically according to Rodent Analgesia SOP 101.
 - 4.5.8.2. Provide moistened food at the bottom of the cage.
 - 4.5.8.3. Administer from 0.2 to 0.5mL/10g body weight of isotonic fluids, subcutaneously.
 - 4.5.8.4. Examine the wound daily for signs of inflammation or infection such as redness, swelling or purulent discharge.
 - 4.5.8.5. Ensure adequate wound closure, presence of sutures or wound clips.
 - 4.5.8.6. Measure body weight.
 - 4.5.9. Remove skin sutures or wound clips after 7 to10 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	NEW VERSION
2018.03.21	3.13 Sterile drapes
2018.03.21	4.1. Document the details of the surgical procedure in the Rodent Procedure Log.
2018.03.21	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	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	4.3.8 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	References in section 5.
2019.03.31	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 2 days.
2019.11.21	4.3.7. Expose the cranium by making a surgical anterior-posterior incision with a scalpel blade (for lesions or injections) or by cutting a circular fold of skin with scissors (for cannula placement).
2019.11.21	4.3.12. Hydrogen peroxide solution can be applied to the skull to facilitate identification of bregma; the sutures will appear white.
2019.11.21	4.3.13. Intracranial injection: 4.3.13.1. Using predefined stereotaxic coordinates, mark on the skull the intended site of injection. 4.3.13.2. Using a hand-held drill, make a single burr-hole in the skull at the injection site. 4.3.13.3. Position the syringe over the burr-hole. 4.3.13.4. Lower the syringe until the needle touches the cortical surface and use this point as "zero" (Z zero). Lower the syringe needle to the desired depth. 4.3.13.5. Inject slowly to avoid an acute increase of intracranial pressure and facilitate diffusion of the fluid. Depending on the total volume injected, this step may take up to 10 minutes. 4.3.13.6. After completing the injection, you may allow 2-5 additional minutes rest time before starting to withdraw the syringe. 4.3.13.7. Withdraw the syringe slowly. 4.3.13.8. It is not necessary to seal the burr-hole.
2019.11.21	4.3.14.1. Using a hand-held microdrill, 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.
2019.11.21	4.3.14.5. Dental acrylic is applied around the cannulae, screws and any exposed cranium to secure the cannulae in place. The dental acrylic should create a strong head cap fixed to the skull. The edges of the head cap should be smooth and should cover all of the bone.
2019.11.21	4.3.15. Implantation of neural implants: 4.3.15.1. 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. 4.3.15.2. Insert stainless steel screws. 4.3.15.3. Make a craniotomy over the target area of the brain according to the predetermined stereotaxic coordinates. 4.3.15.4. Slowly and carefully insert implant(s) into the target area(s) 4.3.15.5. Apply dental acrylic around the electrodes, screws and any exposed cranium to secure in place. The dental acrylic should create a strong head cap fixed to the skull. The edges of the head cap should be smooth and should cover all of the bone.
2020.05.28	3.8 Antiseptic detergent for skin (e.g., chlorhexidine 2% solution or povidone-iodine solution, 70% alcohol, or 2% chlorhexidine in 70% alcohol solution)
2020.05.28	3.10 Heating disc, warming pad or warm-water circulating pad (do not use electric heating pads unless specifically designed for use with laboratory rodents), insulating material (thermal drapes, bubble wrap)
2020.05.28	3.15 Drill, sterile stainless steel screws, sterile cannulae, sterile Hamilton syringe
2020.05.28	3.17 Dry bead sterilizer or cold sterilization agents (e.g. glutaraldehyde) and 70% alcohol (as a rinsing agent)
2020.05.28	4.2.6. Wash the surgical site with 2% chlorhexidine solution or povidone-iodine solution. Be careful not to wet the animal.
2020.05.28	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 4.2.1 and 4.2.2 twice. 4.2.10. Preparation of the surgical site: 4.2.10.1. When using a 2% chlorhexidine in 70% alcohol solution, apply 3 times with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Be careful not to wet a large area on the animal as the evaporation of the solution will lead to heat loss. 4.2.10.2. If using 2% chlorhexidine solution or povidone-iodine solution, first apply 70% alcohol with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Be careful not to wet a large area on the animal as the evaporation of alcohol will lead to heat loss. Then apply 2% chlorhexidine solution or povidone-iodine solution with gauze or swabs in a circular motion, from the center of the surgical site to the exterior. Repeat these steps two more times.
2020.05.28	4.3.5.3. Glad® Press'n Seal® wrap can be used as a surgical drape to cover the animal. As it is transparent, it allows for easier monitoring of the animal.
2020.05.28	4.3.15. 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.
2020.05.28	4.3.17. Recommended suture and wound closure: Tissue: Skin; Suture: Polyamide-nylon, PDS, Vicryl; size: 3-0, 4-0, 5-0, 6-0, Wound clips (Autoclips); 7mm or 9mm; Needle: Cutting, Reverse-cutting Silk suture should be avoided for skin closure as it may cause a local inflammatory tissue reaction and is associated with a higher incidence of wound infection.
2020.05.28	4.4.1. Refer to Rodent Surgery SOP. 4.4.1. Provide a contact heat source to prevent hypothermia. 4.4.2. Frequently monitor the presence of reflexes, the respiratory rate and breathing pattern, and when available, the heart rate. 4.4.3. Adjust the depth of anesthesia according to monitored parameters (presence of reflexes, respiratory rate and breathing pattern, heart rate). 4.4.4. In the case of respiratory arrest, stop anesthesia, administer oxygen and compress the thorax rapidly.

2020.05.28	<p>4.5.1. Refer to Rodent Surgery SOP.</p> <p>4.5.1. Post-operative care begins immediately following surgery, lasts a minimum of 3 days, and extends for up to 10 days.</p> <p>4.5.2. Post-operative animals should be identified with a Post-Procedure cage card.</p> <p>4.5.3. Do not return animals that have not completely recovered to an animal housing room.</p> <p>4.5.4. Observe the animal until it regains righting reflexes; do not leave recovering animal unattended. Observe respiration and coloration of the eyes (for albinos), mucous membranes, and skin.</p> <p>4.5.5. Prevent heat loss and maintain the animal in contact with a heat source or inside a heated cabinet until it regains righting reflexes.</p> <p>4.5.6. Administer oxygen if necessary.</p> <p>4.5.7. For surgeries exceeding 60 minutes, or if there has been significant blood loss, administer an additional 0.2 to 0.5mL/10 g body weight of isotonic fluids, subcutaneously.</p> <p>4.5.8. Monitor animals daily for at least the first 3 days following the surgery. Continue daily monitoring and contact veterinary care staff if recovery is prolonged beyond 3 days. Record supportive care provided on the Post-Procedure cage card.</p> <p>4.5.8.1. Repeat analgesics post-surgically according to Rodent Analgesia SOP 101.</p> <p>4.5.8.2. Provide moistened food at the bottom of the cage.</p> <p>4.5.8.3. Administer from 0.2 to 0.5mL/10g body weight of isotonic fluids, subcutaneously.</p> <p>4.5.8.4. Examine the wound daily for signs of inflammation or infection such as redness, swelling or purulent discharge.</p> <p>4.5.8.5. Ensure adequate wound closure, presence of sutures or wound clips.</p> <p>4.5.8.6. Measure body weight.</p> <p>4.5.9. Remove skin sutures or wound clips after 7 to 10 days.</p>
2020.12.07	4.2.3. Anesthetize the animal according to Rodent Anesthesia SOP (can be done after step 4.2.3).
2021.01.13	4.3.10. Scrape the skull to detach and push aside the periosteum . Wipe the skull surface with sterile swabs to remove blood.
2021.01.13	4.4.4. In the case of respiratory arrest, stop anesthesia, administer oxygen and compress the thorax rapidly between the thumb and index at a frequency of 80-120/min .

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														
6														
7														
8														
9														
10														
11														
12														
13														
14														
Comments/footnotes:														