Carolinas HealthCare System Standard Operating Procedure Manual CANNON RESEARCH CENTER – COMPARATIVE MEDICINE

Title: Anesthesia and Post-Operative Monitoring - Rodent

Purpose:

Current veterinary anesthesia standards of care focus on reducing anesthetic and post-operative morbidity. Adverse changes in normal animal physiology can be detected and corrected early through responsible use of physiologic monitoring equipment and trained personnel dedicated to anesthesia and post-operative monitoring.

The purpose of this SOP is to serve as a guideline for anesthesia monitoring during surgery and sedation as well as post-operative monitoring. This is not intended to be an inclusive tutorial on all possible methods and all equipment available for monitoring. Monitoring procedures specifically detailed in an approved Institutional Animal Care and Use Committee (IACUC) protocol must be followed as well as instruction provided by the Attending Veterinarian.

Administered anesthesia, sedation, and analgesics should be in accordance to the Rodent Anesthesia and Sedation SOP and as outlined in an approved IACUC protocol or at the instruction of the Attending Veterinarian.

Responsibilities:

Attending Veterinarian (AV): The AV is responsible for the oversight and training of personnel performing rodent anesthesia and sedation.

Vivarium Supervisor: The Supervisor is responsible for the oversight, training, and verification of training and compliance of animal handling personnel performing rodent anesthesia and sedation.

Animal Handling Personnel: Technicians must be properly trained to follow procedures described in this SOP.

Research Staff: The Principal Investigator (PI) is responsible for ensuring that appropriate anesthesia, monitoring, and analgesia is provided for all animals undergoing surgical or sedated procedures; that all members of the research team have the appropriate training and experience; and that only anesthesia and sedation described in the IACUC protocol is administered. PIs are expected to purchase anesthesia and monitoring equipment corresponding to the complexity and potential anesthetic complications associated with their surgical research model.

Definitions/Acronyms:

1. Attending Veterinarian (AV): a person who has graduated from a veterinary school accredited by the American Veterinary Medical Association's Council on Education, or

has a certificate issued by the American Veterinary Medical Association's Education Commission for Foreign Veterinary Graduates, or has received equivalent formal education as determined by the Administrator; has received training and/or experience in the care and management of the species being attended; and who has direct or delegated authority for activities involving animals at a facility subject to the jurisdiction of the Secretary.

- 2. Personal protective equipment (PPE): protective clothing, other garments, or equipment designed to protect the wearer's body from injury, infection, or exposure.
- 3. Anesthesia: temporarily induces loss of sensation with or without loss of consciousness. Does NOT provide pain relief.
- 4. Analgesia: provides pain relief without loss of consciousness.
- 5. Sedation: a mild degree of central nervous system depression in which the patient is awake but calm. Sedation is NOT sufficient for surgical procedures.
- 6. Sterile (aseptic): free from bacteria or other living microorganisms.

Materials:

- 1. Rodent Survival (or Non-Survival) Anesthesia Monitoring form
- 2. Rodent Surgery Record/Rodent Post-Operative Record cage cards (survival surgeries)
- 3. Species appropriate scale
- 4. Heating source
- 5. Rodent surgical monitor
- 6. Pulse oximeter
- 7. Personal Protective Equipment (see Personal Protective Equipment SOP)

Procedures

1. Recovery Surgical Anesthesia Expectations

- 1.1. Procedures must meet current veterinary anesthesia standards of care.
- 1.2. PIs are expected to purchase anesthesia and monitoring equipment corresponding to the complexity and potential anesthetic complications associated with their surgical research model.

- 1.3. Researchers are expected to maintain aseptic technique while providing appropriate anesthetic monitoring. Typically, this requires one person dedicated to surgery and a separate person dedicated to anesthesia monitoring.
- 1.4. Use of room air as a carrier for anesthetic inhalants is highly discouraged. If not using oxygen for a carrier, a pulse oximeter should be used to monitor blood oxygenation saturation throughout the surgical procedure.

2. Anesthesia Monitoring

2.1. Preparation

- 2.1.1. Examine general animal health prior to anesthesia. The AV should be consulted if there are any health concerns (e.g., poor body condition, ruffled hair coat, hunched posture, diarrhea, etc.).
- 2.1.2. Once the rodent is anesthetized, the anesthetic depth must be evaluated prior to starting the surgical procedure. Appropriate anesthetic depth is determined by absence of painful response, such as pedal or toe pinch.
- 2.2. Monitoring every 10-15 minutes throughout the anesthetic event is recommended.
 - 2.2.1. Cardiovascular System: Tail, foot, tongue or ear color can be monitored for pale or blue membranes which is indicative of decreased blood volume, decreased perfusion or respiratory distress.
 - 2.2.2. Respiratory System: Small rodents have respiratory rates too quick to accurately count. Instead, an assessment of the breathing pattern should be made. (e.g., regular vs. irregular breaths).
 - 2.2.3. Body Temperature: Rodents have a high body surface area to mass ratio, which causes them to heat up quickly when warmed and lose body heat quickly when chilled. As a result, rodents are prone to overheating when high temperature heating systems are used and hypothermia if supplemental heat is not provided. Both, thermal excess and deficit, can quickly lead to serious injury or death.
 - 2.2.3.1. During recovery, heating pads can be placed under the rodent cage so half of the cage is on the pad and half off the pad. This will allow animals to escape the heat source if they become too hot.
 - 2.2.3.2. Warming devices should provide gentle heat only (maximum of $102^{\circ}F$). A thermometer can be placed next to the supplemental heat source to verify temperatures are < 102.
 - 2.2.3.3. An insulated pad or folded drape must be placed between the animal and the heat source.

- 2.2.3.4. AVOID commercial human electric heating pads: Maximum temperatures are not well regulated and uneven surface temperature gradients exist risking thermal injury burns to the animal.
- 2.2.3.5. Heating lamps are discouraged since they are a fire hazard risk and the maximum temperature is difficult to regulate. However, heat lamps can be used as long as they are placed an appropriate distance away from the animal to ensure thermal burns do not occur and animals are able to move away from the heated portion of the cage.
- 2.2.3.6. Recommended warming devices for rodents
 - Circulating water blanket (Gaymar®)
 - Rodent isothermal warming pads (Braintree Scientific®)
 - Commercial homeo-thermic rodent heating pads with thermostat control to regulate maximum temperature
 - Commercial insulating pouches and surgical drapes (Spacedrapes®)
 - Commercial rodent incubators

2.3. Anesthetic Depth

- 2.3.1. Adequate anesthetic depth must be evaluated during anesthetic period.
- 2.3.2. Indicators of anesthetic depth include loss of stimulation (includes toe, ear, or tail pinch), loss of mandibular jaw tone, and absent palpebral reflex (or blink reflex).

3. Post-Operative/Post-Procedural Monitoring

- 3.1. Animals must be visibly observed and monitored every 10-15 minutes during recovery from anesthesia until the animal is ambulatory.
- 3.2. Recover animals in the surgery area so they can be appropriately monitored during the recovery period.
- 3.3. Rodents should be housed individually until completely recovered to avoid cannibalism by cage mates.
- 3.4. Recover animals in a bare cage or on top of a blue pad (or similar barrier) to prevent aspiration of bedding. Do not place anesthetized animals on loose bedding material.
- 3.5. Monitoring parameters and thermal supplementation should be continued throughout the recovery period. Please refer to Section 2.2.3.6 for more information on monitoring and appropriate thermal support devices.

- 3.6. Nutritional support is critical following anesthesia and should be provided as soon as the animal is recovered. Moist chow, regular chow, or diet gel should be provided on the cage floor to encourage eating as soon as possible.
- 3.7. Post-operative medications including analgesics, antibiotics and/or anesthetic reversals should be administered during the early recovery period and according to the approved IACUC protocol or the advice of the AV.
- 3.8. A yellow-bannered Rodent Surgery Record/ Rodent Post-Operative Record cage card should be placed on the cage when the fully recovered animal is returned to the animal housing room. See Section 4 for record keeping.
- 3.9. The research staff must examine all post-surgical animals at least once a day for 7-10 days or until skin sutures or wound clips are removed. Daily observations must be recorded on the Rodent Surgery Record/Rodent Post-Operative Record cage card (see Section 4).

4. Record Keeping

- 4.1. Survival surgical anesthetic monitoring should be documented using a Rodent Survival Anesthesia Monitoring form (Appendix A). The following details should be provided:
 - 4.1.1. IACUC protocol number and PI name.
 - 4.1.2. Date of surgery, species, animal ID, animal date of birth (DOB), animal sex, animal body weight, surgery start time, and surgery end time.
 - 4.1.3. Briefly describe the surgical procedure, surgeon(s), surgery room, and the name of any additional assistance (e.g., technical staff, anesthetist).
 - 4.1.4. Record all premedication, induction agents, and any other anesthetic agents: drug name, dose (mg/kg), concentration (mg/ml), volume (ml), route, time administered, and initials of drug administrator.
 - 4.1.5. Initial that ophthalmic lubricant has been applied.
 - 4.1.6. Indicate any shaved/aseptically prepped area and initial.
 - 4.1.7. For inhalant agents, record agent name, induction flow rate (%), maintenance flow rate (%), carrier gas (oxygen) flow rate (L/min), start time, and initial.
 - 4.1.8. Indicate method used to verify that the proper surgical level of anesthesia was met prior to the first incision and initial.
 - 4.1.9. Monitoring parameters (see section 2.2) should be recorded every 10-15 minutes and should include the following details: time, mucous membrane or skin color,

- respiratory rate or pattern, and temperature (if accessible, otherwise write "NA").
- 4.1.10. Post-surgically, record recovery location, recovery start time, sternal time, and time returned to the animal housing room.
- 4.1.11. Any additional comments may be recorded in the "Notes" section.
- 4.2. Post-operatively a yellow-bannered Rodent Surgery Record/Rodent Post-Operative Record cage card should be placed on the cage and the following details should be provided:
 - 4.2.1. One card should be placed per animal, if there are multiple animals in a cage, then multiple cards should be placed.
 - 4.2.2. On the "Rodent Surgery Record" side (Appendix B, Front) record room/cage location, date placed, surgeon, surgical procedure, PI name, IACUC protocol number, animal ID, and pre-surgical body weight, administered drug information (category, name, dose, route, time), and recovery notes (include recovery time).
 - 4.2.3. On the "Rodent Post-Operative Record" side (Appendix B, Back) the following should be recorded:
 - 4.2.3.1. Date: Animals should be monitored 7-10 days post-operatively.
 - 4.2.3.2. Time: Time of observation.
 - 4.2.3.3. Surgical Site: Either describe or use the letter key listed on the card.
 - 4.2.3.4. Pain: Either note pain presence as Y (yes) or N (no) or grimace scale scores (Appendix C and D).
 - 4.2.3.5. Other Observations: Describe and/or use the number key listed on the card.
 - 4.2.3.6. Drugs Given: Include name, dose, route.
 - 4.2.3.7. Initial: Observer's initials.
- 4.3. Non-survival anesthetic monitoring for procedures in excess of 15 minutes duration should be documented using a Rodent Non-Survival Anesthesia Monitoring form (Appendix E).
 - 4.3.1. Follow steps 4.1.1 through 4.1.9.
 - 4.3.2. Euthanasia time, reason, and initials of individual performing euthanization should be recorded (additional drug information should be provided to include details

outlined in 4.1.4).

- 4.3.3. Any additional comments may be recorded in the "Notes" section.
- 4.4. Short-term anesthesia administration (less than 15 minutes) need not be recorded using a Rodent Survival Anesthesia Monitoring form. For these cages, a purple "Notes to the Vivarium" card just be placed noting the procedure performed.
- 4.5. Surgical details are not required; however, it is recommended that specific surgery details be documented in a laboratory notebook.
- 4.6. All records must be kept for 3 years.

References

- 1. Guide for the Care and Use of Laboratory Animals (The Guide).
- 2. Office of Laboratory Animal Welfare IACUC Guidebook (OLAW).
- 3. American College of Laboratory Animal Medicine Guidelines for the Assessment and Management of Pain in Rodents and Rabbits (ACLAM).

Approved January 2018

Appendix A

IACUC P PI:	Protocol: Rodent Survival Anesthesia Monitoring									Carolinas HealthCare		
Date:		Spec	ies:			A	nimal ID:			Sta	art Time:	
DOB:				s			ody Weight	t:	_		d Time:	
Surgical Pro						-						
Surgery Ro												
Technical S	taff/Anesthe	etist:										
	Injectable l	Pre-Me	dicati	on, In				ther D	rugs	(In	ijectable)	
Dı	Drug Name			Dose (mg/kg)		c. Volume L) (mL)	Route			Time	Initials	
Eye ointmet Appropriat	nt/lube (initi e areas shav Gas Name	als): ed/prep	oped:	In % I	ion: halant / Flow iction	Ane		02	Flov	v	Start Time	mitto
										_		
Proper Sur	gical Level o	of Anest	hesia	Verif	<mark>ied</mark> : Met	hod	i				Initials:	
Anesthesia	and Monit	oring:	Recor	d vita	l signs a	mir	nimum of ev	ery 10-1	15 m	imut	tes	
Time Color ¹		Respira (rate/pa		Temp (°F)³			Time	Color ¹	Color ¹		spiration ² te/pattern)	Temp (^e F) ³
									_			
Mucous membran	es should be pink (i	f inaccessible	e, use pa	w pad and	d ear color); ²	Respi	rations should be	regular and	deep;	4f usir	ng rectal probe	otherwise "NA"
				Post-	Surgica	l M	onitoring:					
Recovery Location:						$\overline{}$	Recovery Start Time:					
Sternal Time:						I	Time Returned to Housing Room:					
Notes:												

Appendix B

Front

RODENT SURGERY RECORD										
Room/Cage:	PI/Protoco	ol #:								
Date Placed:	Animal ID	:								
Surgeon:	Pre-Surgio	Pre-Surgical Body Weight:								
Surgical Procedure:										
Surgical Drugs Administered:										
	Drug	Dose (mg)	Route	Time						
Anesthetic										
Dra a mutius Analassis										
Preemptive Analgesic										
Other										
Recovery notes (include times):										
	L	!!								
			(Post-Operative R	ecord on reverse)						

Back

Day	Time	Surgical Site describe, and/or use	Painful? Y/N, grimace score	Other Observations describe, and/or use	Drugs Given	Initial			
		letter key		number key	name, dose, route				
				gical site and animal con					
		an, dry, intact		1 - animal is bright, alert					
B- incis	on is slig	htly red, dean, dry, int	act	2 - animal is quiet, alert, and responsive					
C - incis	ion is ab	normal, please describ	e•	3 - animal is lethargic and less responsive*					
		*Contac	t Veterinarian		(Surgery Record o	n rever			

Appendix C

The Rat Grimace Scale

Orbital tightening

- Closing of the eyelid (narrowing of the orbital margin)
- A wrinkle may be visible around the eye

Nose/cheek flattening

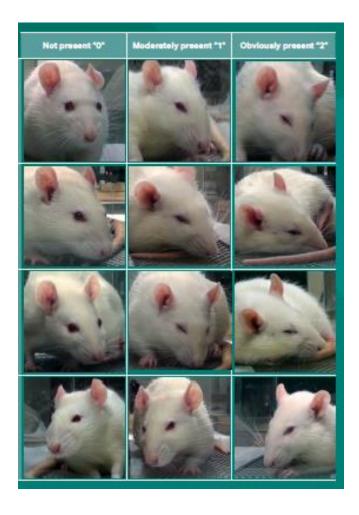
- Flattening and elongation of the bridge of the nose
- Flattening of the cheeks

Ear changes

- Ears curl inward and are angled forward to form a "pointed" shape
- Space between the ears increases

Whisker change

- Whiskers stiffen and angle along the face
- Whiskers may "clump" together
- Whiskers lose their natural "downward" curve



Appendix D

The Mouse Grimace Scale

Orbital tightening

- Closing of the eyelid (narrowing of the orbital margin)
- A wrinkle may be visible around the eye

Nose bulge

- Bulging on the bridge of the nose
- Vertical wrinkles on the side of the nose

Cheek bulge

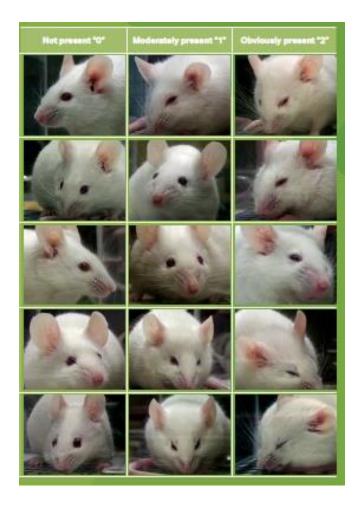
• Bulging of the cheeks

Ear position

- Ears rotate outwards and backwards, away from the face
- Space between the ears increases

Whisker change

- Whiskers are either pulled back against the cheek or pulled forward and "stand on end"
- Whiskers may "clump" together
- Whiskers lose their natural "downward" curve



Appendix E

IACUC P PI:	UC Protocol: Rodent Non-Survival Anesthesia Monitoring										
Date:		Species	:		Aı	nimal ID:		St	art Time:		
DOB:		Sex:				ody Weight:		Eı	End Time:		
Surgical Pro Surgeon(s):											
Surgery Ro											
Technical S	taff/Anesthe	etist:									
Inject	table Pre-M	edication,	Inductio	n Agen	ts, E	uthanasia a	nd Othe	r Dr	ugs (Injec	table)	
Dı	Drug Name			Dose Conc ng/kg) (mg/m		Volume (mL)	Route		Time	Initials	
								_			
								+			
								_			
Appropriate	e areas shav	ed/preppe						-		1	
				halant .	_	sthesia % Flow	O ₂ Flo		Stout		
	Gas Name			% Flow Induction		aintenance	_		Start Time	Initials	
Proper Surg	gical Level o	of Anesthes	sia Verif	<mark>ied</mark> : Me	thod		I		_Initials: _		
A	nesthesia ar	nd Monitor	ring: Re	cord vit	al si	gns a minim	um of eve	ry 10)-15 mimut	es	
	Color ¹		² Ten			Time				Temp (°F) ³	
					\perp						
Mucous membrane										otherwise "NA"	
Euthanasia: *Note: Agent dos	ing information	; I should go in ini	nitials: _ ectable drus	gs table	Rea	ison:					
Notes:											