UNIVERSITÉ LAVAL **Direction des services vétérinaires**	**Standard Operating Procedure**
Subject: Analgesia and anesthesia in rodents	Number: A-1
Scope: A directive from the Direction des services vétérinaires to users and staff of Université Laval animal facilities (campus and affiliated research centres).	
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Purpose: Describe the analgesia and anesthesia procedures for rodents.	Version 8

General Considerations

- If pain is anticipated, administer analgesia before the painful stimulus. A procedure that is painful in humans is considered painful in animals as well. When in doubt, the animal's welfare takes priority.
- Where the experimental protocol does not allow for an analgesic, follow SOP ETH-9 Non-utilization of an Analgesic (PNF ETH-9 Non-utilisation d'un analgésique).
- Using injectable anesthetics for long-term procedures is not recommended due to a heightened risk of overdose. A decision to use anesthesia by injection with booster doses must first be justified to CPAUL.
- When animals experience pain and distress, it may affect the experimental results.
- Animal users and staff must be able to recognize signs of pain and distress in animals.
- Animals can be anesthetized via inhalation of anesthetic gases or by injection of anesthetics. Inhalation is the method of choice, given that injections are less safe and their effects may vary between animals.
- To prepare drug combinations, consult SOP A-14 Dilution and storage of drugs (PNF A-14 Dilution et entreposage des drogues).
- Use a heating mat throughout the anesthesia procedure to avoid hypothermia. Electric heating mats can be used under cages only.
- The following equipment is safe to use for maintaining an animal's temperature during anesthesia: circulating water mat, Deltaphase®, far-infrared pad, or an electric heating plate with a rectal thermometer for precise heat adjustment (limit the device's temperature to a maximum of 41°C and ensure the animal's body temperature does not exceed 39°C).

- Always apply ophthalmic ointment after loss of consciousness to prevent corneal dryness, regardless of the anesthesia method used. Reapply ointment during lengthy procedures.

Definitions

- Analgesia: reduction or suppression of sensitivity to pain
- Anesthesia: general or partial loss of consciousness or local loss of sensation
- Local block: local infiltration anesthesia
- Sternal recumbency: animal lying on its stomach
- Induction: the first stage of anesthesia, which involves putting the animal to sleep
- IP: intraperitoneal injection
- SC: subcutaneous injection
- Sedation/tranquilization: calms and reduces the anxiety felt by the animal
- CRT: capillary refill time

Procedures

Analgesia

- Three main classes of analgesics are generally used: opioids, NSAIDs (non-steroidal anti-inflammatory drugs), and local anesthetics. Multimodal analgesia is preferred (opioids and/or NSAIDs and/or a local anesthetic).
- The frequency of administration and the minimum duration of analgesia depend on the drug used and the procedure's invasiveness (see Table 1 and Table 2).
- Local anesthetics are used for minor procedures or to block sensation within a limited area (see Table 3). Local blocks are quick and easy to inject, inexpensive, and very effective in countering the onset of postoperative pain. For this reason, they are mandatory for all surgeries, if not contraindicated by the protocol.
- Systemic toxicity (including seizures or even death) can be avoided by not exceeding maximum doses. Care should also be taken not to inject into the bloodstream.
- NSAIDs are not recommended in very young animals. In cases where unweaned rodents require analgesics, local anesthetics and opioids are indicated.

Table 1: Analgesic protocols for mice

Level of pain associated with the procedure	Analgesic protocol	Time of injection	Number of doses	Analgesic agent	Dose (mg/kg)	Route
1 (Mild) Minimum 24 hours of analgesia (e.g., subcutaneous pump, castration, etc.)	A	Pre-op	1	Buprenorphine ♣	0.05	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		Immediately post-op	1	Meloxicam**	1	SC
	B	Pre-op	1	Meloxicam**	1	SC
		Pre-op	1	Lido / Bupi	See Table 3	
	C	Pre-op	1	Buprenorphine ♣	0.05	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		8–12 hours after the 1st injection	1	Buprenorphine ♣	0.05	SC
2 (Moderate) Minimum 48 hours of analgesia (e.g., intra-abdominal pump, ovariectomy, stereotaxic surgery, etc.)	A	Pre-op	1	Buprenorphine ♣	0.05	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		Immediately post-op and 24 hours later	2	Meloxicam**	1	SC
	B	Pre-op	1	Meloxicam**	1	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		24 hours after the 1st injection	1	Meloxicam **	1	SC
	C	Pre-op	1	Buprenorphine* ♣	0.05	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		At 8–12 hours	3	Buprenorphine* ♣	0.05	SC
3 (Severe) Minimum 72 hours of analgesia (e.g., thoracotomy, laminectomy, extensive abdominal surgery, etc.)	A	Pre-op	1	Buprenorphin * ♣	0.1	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		Immediately post-op and 24 and 48 hours later	3	Meloxicam **	1	SC
	B	Pre-op	1	Meloxicam**	1	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		Immediately post-op, then at 8-12 hours	6	Buprenorphine* ♣	0.1	SC

* One pre-op injection of buprenorphine SR (0.5–1 mg/kg) can replace all buprenorphine injections.

♣ Controlled drug

** A 20 mg/kg dose of carprofen can replace meloxicam.

Table 2: Analgesics commonly used in rats

Level of pain associated with the procedure	Analgesic protocol	Time of injection	Number of doses	Analgesic agent	Dose (mg/kg)	Route
1 (Mild) Minimum 24 hours of analgesia (e.g., subcutaneous pump, castration, etc.)	A	Pre-op	1	Buprenorphine ✱	0.01	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		Immediately post-op	1	Meloxicam**	1	SC
	B	Pre-op	1	Meloxicam**	1	SC
		Pre-op	1	Lido / Bupi	See Table 3	
	C	Pre-op	1	Buprenorphine ✱	0.01	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		8–12 hours after the 1st injection	1	Buprenorphine ✱	0.01	SC
2 (Moderate) Minimum 48 hours of analgesia (e.g., intra-abdominal pump, ovariectomy, stereotaxic surgery, etc.)	A	Pre-op	1	Buprenorphine ✱	0.01	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		Immediately post-op and 24 hours later	2	Meloxicam**	1	SC
	B	Pre-op	1	Meloxicam**	1	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		24 hours after the 1st injection	1	Meloxicam**	1	SC
	C	Pre-op	1	Buprenorphine* ✱	0.01	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		At 8–12 hours	3	Buprenorphine* ✱	0.01	SC
3 (Severe) Minimum 72 hours of analgesia (e.g., thoracotomy, laminectomy, extensive abdominal surgery, etc.)	A	Pre-op	1	Buprenorphine* ✱	0.05	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		Immediately post-op and 24 and 48 hours later	3	Meloxicam**	1	SC
	B	Pre-op	1	Meloxicam**	1	SC
		Pre-op	1	Lido / Bupi	See Table 3	
		Immediately post-op, then at 8-12 hours	6	Buprenorphine* ✱	1	SC

* One pre-op injection of buprenorphine SR (1–1,2 mg/kg) can replace all buprenorphine injections.

✱ Controlled drug

** A 5 mg/kg dose of carprofen can replace meloxicam.

Table 3: Local anesthetics (combination of lidocaine and bupivacaine*)

	Administration method	Local infiltration (SC)
Mouse	Maximum dose per animal	7 mg/kg (lidocaine) 3,5 mg/kg (bupivacaine)
	Maximum volume per animal	0.08 ml/10 g
	Recommended volume for a 1 cm incision	About 0.05 ml
	Recommended volume for stereotaxic surgery	0.03 ml in each ear (if ear bars are used)
Rats	Maximum dose per animal	7 mg/kg (lidocaine) 3.5 mg/kg (bupivacaine)
	Maximum volume per animal	0.25 ml/100 g
	Recommended volume for a 1 cm incision	About 0.1 ml
	Recommended volume for stereotaxic surgery	0.05 ml in each ear (if ear bars are used)

* Always combine lidocaine and bupivacaine to achieve a rapid (<10 minutes) and long-lasting (3–6 hours) local anesthesia.

Sedation

- A number of agents alone or in combination may be used during non-invasive procedures such as dental exams, x-rays, etc. Specifically select these agents to meet the procedure's requirements: sedation with or without relaxation, duration of sedation, side effects, etc.
- Working with the veterinarians, select the sedative agent(s) for the experiment you will carry out.
- Administer the required doses and volumes as specified in the experimental protocol.

Anesthesia

Equipment preparation

- Turn on the heating mat about 10 minutes before starting.
- Check the anesthesia machine for leaks, calibration date, and oxygen level. For inhalation anesthesia, check the isoflurane level. Make sure the gas collection system is working properly.
- Have a clean induction chamber on hand.

Premedication

- Premedication is not routinely given to rodents.
- Being nervous by nature, Guinea pigs can be premedicated before induction using 0.5 mg/kg of acepromazine intramuscularly.

Animal preparation

- Guinea pigs: Fast the animal 1 to 2 hours before anesthesia to eliminate its food intake. Before induction, gently rinse its mouth with 10 ml of water to remove any food remaining in its cheeks.
- Perform a physical exam and measure the animal's weight the same day.
- Administer warmed Lactated Ringers Solution (LRS) by subcutaneous injection during anesthesia that lasts more than 10 minutes or during a surgical procedure.

Table 4: LRS volumes to administer preoperatively and hourly

Species	LRS volume hourly	Administration route
Mice	0.1 ml/10 g/h (10 ml/kg/h)	SC
Rats	0.5 ml/100 g/h (5 ml/kg/h)	SC
Hamsters	0.05 ml/10 g/h (5 ml/kg/h)	SC
Guinea pigs	0.5 ml/100 g/h (5 ml/kg/h)	SC

- Administer the warmed dose of preoperative analgesia, as per the protocol, during the surgical procedure. Calculate an onset of action time of approximately 30 minutes; depending on how long you require to prepare the surgical site, the injectable anesthetic can be administered before or after induction.
- The local block must be administered after induction. Allow for a 3-minute onset of action. The volume administered must adequately cover the incision area without causing tissue distortion.

Isoflurane inhalation anesthesia

Induction

- Place the rodent in a clean induction chamber connected to the anesthesia machine. Adjust the isoflurane to 3-4% and the oxygen flow rate to 0.8-1.5 L/min.
- Apply ophthalmic ointment to the animal's eyes.
- Intubate the animal, if necessary.

Maintenance

- Transfer the animal to a Bain circuit with a mask or connect the Bain circuit directly to the endotracheal tube.
- Adjust the oxygen flow meter to 500 ml/min.

Note: If multiple connections are used on the same device, increase the oxygen flow rate.

- Adjust the isoflurane vaporizer to 1.5–2% or higher depending on the desired depth of anesthesia.
- Make sure the level of anesthesia is adequate for the procedure (see Table 9).
- Decrease or increase the isoflurane level as needed.
- Reapply ophthalmic ointment every 30 minutes.
- Re-administer the warmed LRS throughout the procedure, following the hourly volumes to administer (see Table 4).

Anesthesia by injection

Ketamine/xylazine or ketamine/ xylazine/acepromazine combination

Table 5: Anesthesia with a ketamine-xylazine combination

Species	Ketamine ♣ (mg/kg)	Xylazine (mg/kg)	Volume to administer	Administration route
Mouse	100	10	10 ml/kg 0.1 ml/10 g	IP
Rat	80	10	1 ml/kg 0.1 ml/100 g	IP
Hamster	150	10	2 ml/kg 0.2 ml/100 g	IP
Guinea pig	40	5	1 ml/kg 0.1 ml/100 g	IP

♣ Controlled drug: Fill out the controlled drug registry to use ketamine.

Table 6: Anesthesia with a ketamine-xylazine-acepromazine combination

Species	Ketamine * (mg/kg)	Xylazine (mg/kg)	Acepromazine (mg/kg)	Volume to administer	Administration route
Mice	50	10	1.7	10 ml/kg 0.1 ml/10 g	IP
Rat	50	5	1	1 ml/kg 0.1 ml/100 g	IP
Hamster	50	5	1	1 ml/kg 0.1 ml/100 g	IP
Guinea pig	25	2.5	1	1 ml/kg 0.1 ml/100 g	IP

* Controlled drug: Fill out the controlled drug registry to use ketamine.

Induction

- Administer the first injection intraperitoneally.

Maintenance

- You must provide oxygen to the animal during the procedure.
- If necessary, administer a booster dose intraperitoneally after 20 to 30 minutes. The interval may vary from species to species and from animal to animal. The first booster dose should be a half dose of ketamine alone; the next will be a half dose of the original ketamine-xylazine combination or a quarter dose of the ketamine-xylazine-acepromazine combination, and so forth:

Table 7: Booster doses for anesthesia with a ketamine-xylazine combination

Species	Ketamine* only (mg/kg)	Ketamine */ xylazine (mg/kg)	Volume to administer	Administration route
Mouse	50	50 / 5	5 ml/kg 0.05 ml/10 g	IP
Rat	40	40 / 5	0.5 ml/kg 0.05 ml/100 g	IP
Hamster	75	75 / 5	1 ml/kg 0.1 ml/100 g	IP
Guinea pig	20	20 / 2.5	0.5 ml/kg 0.05 ml/100 g	IP

* Controlled drug: Fill out the controlled drug registry to use ketamine.

Table 8: Booster doses for anesthesia with a ketamine-xylazine-acepromazine combination

Species	Ketamine* only (mg/kg)	Volume to administer	Ketamine* / xylazine / acepromazine (mg/kg)	Volume to administer	Administration route
Mouse	50	5 ml/kg 0.05 ml/10 g	12.5 / 2.5 / 0.425	2.5 ml/kg 0.025 ml/10 g	IP
Rat	40	0.5 ml/kg 0.05 ml/100 g	12.5 / 1.25 / 0.25	0.25 ml/kg 0.025 ml/100 g	IP
Hamster	75	1 ml/kg 0.1 ml/100 g	12.5 / 1.25 / 0/25	0.25 ml/kg 0.025 ml/100 g	IP
Guinea pig	20	0.5 ml/kg 0.05 ml/100 g	6.25 / 0.625 / 0.25	0.25 ml/kg 0.025 ml/100 g	IP

* Controlled drug: Fill out the controlled drug registry to use ketamine.

- Reapply ophthalmic ointment every 30 minutes.
- Re-administer the warmed LRS throughout the procedure, following the hourly volumes to administer (see Table 4).

Anesthesia monitoring

- Never leave an anesthetized animal unattended.
- Monitor vital signs continuously from the time of loss of consciousness until full recovery (see Table 9 and Table 10).

Table 9: Monitoring the depth of anesthesia

Depth of anesthesia	Heart rate	Respiratory rate	CRT	Mucous membrane colour	Withdrawal○/palpebral reflexes	Average temperature (°C)
Light	High	Rapid	< 2 sec	Pink	Present / present	Normal
Moderate	Regular	Slowed	< 2 sec	Pink	Slight / slight	Normal
Deep*	Slowed	Deep and regular	< 2 sec	Pink	Absent / absent	Normal to low
Very deep	Slow	Slow and difficult	> 2 sec	Pale or blue	Absent / absent	Low

* Target depth of anesthesia for a surgical procedure.

○ Check the withdrawal reflex by pinching the skin between the toes with Adson forceps (mouse teeth).

Table 10: Normal values in awake rodents

Species	Temperature (°C ± 0.5)	Average ♥ rate	Average respiratory rate
Mouse	37,5	470	138
Rat	37,0	350	92
Hamster	36.2 – 37.5	346	74
Guinea pig	37.2 – 39.8	305	73

These values tend to decrease in an animal under general anesthesia.

Recovery

- Turn off the isoflurane vaporizer, if used, and provide pure oxygen to the animal for about 30 seconds.
- If xylazine was used, administer a dose of atipamezole (1 mg/kg SC) to antagonize its effects and speed up recovery.
- Place the animal in its cage. The recovery area must be clean and quiet.
- Continue to provide heat.
- Administer an additional dose of warmed LRS (see Table 4) if it has been more than 50 minutes since the last dose. Provide wet food in the bottom of the cage and a bottle of water to facilitate recovery.
- When using injectable anesthesia, it is important to allow for an extended recovery time.

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SOP Revision History		
Version 2	January 11, 2013	Added a 20-minute onset of action for the preoperative analgesia injection. Removed acetaminophen from frequently used analgesics.
Version 3	June 10, 2013	Added a definition for "local block" and clarified its use. Clarified recommended types of analgesia based on the expected pain level. Clarified the volumes of subcutaneous fluids injected.
Version 4	March 26, 2014	Modified the local anesthetics table. Modified the analgesia tables (Tables 1 & 2). Added ice anesthesia for baby rats and mice less than 6 days old.
Version 5	January 12, 2016	Modified the carprofen dose for mice. Added buprenorphine SR to Tables 1 and 2. Added clarifications on the heating mat to be used.
Version 6	July 11, 2016	Added clarifications on the frequency of subcutaneous fluid administration.

Version 7	July 3, 2018	Added the xylazine antagonist, atipamezole. Modified the dose of buprenorphine SR for rats. Removed the lidocaine/bupivacaine volume per incision site.
Version 8	December 20, 2019	Additions to General Considerations Relocated the analgesia tables (1, 2, and 3). Removed option 3C from Tables 1 and 2. Clarified local block injection volumes. Removed 0.9% saline as an acceptable fluid and simplified Table 4. Added the doses of the injectable combination of ketamine-xylazine-acepromazine (Tables 6 and 8). Added the requirement to provide oxygen during anesthesia by injectable anesthetics.