LAB_025 Rodent Anaesthesia - Injectable Agents

I. OBJECTIVE

To describe the procedure for monitoring and supporting rodents during anaesthesia within UQBR facilities.

NB: The use of (*) indicates this statement is dependent on the facility procedures
NB: The use of (**) indicates this statement is dependent on AEC Approvals

II. COMMENTS / RECOMMENDATIONS

- Oxygen should always be provided to rodents under anaesthesia.
- Variability in response to injectable anaesthesia is common.
- The anaesthetic period should not exceed 3 hours in duration. Long anaesthesia requires additional consideration of animal support, particularly related to maintaining body temperature, fluid status, and eye protection.
- Workstations must allow rodents to be in your visual field for the duration of their anaesthetic period.
- An assistant is strongly recommended to be present for all surgical procedures.
- Wherever possible anaesthetic monitoring equipment should be used to supplement manual/ visual monitoring (e.g. use of specialised blood pressure, pulse oximetry or respiratory rate monitoring equipment).
- Complications associated with anaesthetic and surgical procedures should be are referred to the UQBR veterinarians for support (see LAB_022 UQBR Veterinary Care Protocol).

In relation to human safety:

- Facility and procedure appropriate PPE use is essential when handling laboratory rodents
- All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form. This procedure has particular risks of:
  - needle stick and mouse bite injury – take appropriate care
  - splash back into the face or eyes when injecting – wear appropriate PPE
  - musculoskeletal injury when performed regularly – consider suitable ergonomic design wherever possible
- In the event of a spill follow facility emergency spill procedures relative to SDS details.

III. EQUIPMENT

- PPE *
- Needles (25-29g)
- Syringes (zero dead space and twice the size of injection volume)
- Anaesthetic agents** (as approved by AEC)
- Dose calculation charts (see Appendices)
- Eye care lubricant (sterile and aqueous e.g. Lacrilube®)
- Weighing scales

- Heating equipment (must include active heating e.g. a heat mat)
- Timing device
- Anaesthetic Monitoring Record (see Appendices)
- Oxygen delivery system, if available (e.g. inhalant anaesthetic unit, without the vaporiser turned on)
- Anaesthetic monitoring equipment (**) (e.g. Physiosuite®, Somnosuite®, pulse oximeter, rectal temperature probe)

IV. PREPARATION

1. Perform a pre-anaesthetic assessment of the animals' general physical condition (including measurement of pre-anaesthetic body weight). If the animal appears unwell it **should not** be anaesthetised.
2. Label the Anaesthetic Monitoring Record with relevant details (e.g. rodent identification, physical condition and procedure to be performed)
3. Ensure the heating equipment in both the procedure and recovery areas is turned on in advance and all anaesthetic equipment is set up within the appropriate workspace (including oxygen delivery) (*)
4. Ensure the timing device (timer, watch, wall clock) is accessible within the workspace (*)
5. Ensure the correct dose of anaesthetic drug is drawn up ready to inject (relative to individual body weights).
6. Clearly label all anaesthetic drugs.

NOTE: One syringe should contain the anaesthetic dose for only one rodent. Multiple anaesthetic doses in one syringe presents potential for inaccuracies (can lead to injection errors) and is not consistent with aseptic technique (can lead to contamination).

V. PROCEDURE

1. Restrain and inject rodent as per LAB_028 Injections – Intra-peritoneal in Mice, Rats and Neonates.
2. Place the rodent into an individual cage in which it can be monitored (this may be the home cage if housed individually) and record the "start-time" within the Anaesthetic Monitoring Record.
3. Once loss of the righting reflex (LORR) is observed (see table 1), gently collect the rodent and place it onto the pre-warmed workspace. Record time at which LORR was observed.
4. From LORR, continuously monitor the rodent's vital signs and anaesthetic depth until anaesthetic recovery. Observations should be recorded every 1-15 minutes (within the Anaesthetic Monitoring Record).
5. Apply eye lubrication.
   This should be done with clean technique, using a small amount of eye lubricant applied to a fresh cotton tip. When applying the lubricant to the eyeball's surface the cotton tip itself should not actually contact the eyeball.
6. Apply any anaesthetic monitoring equipment to the rodent (e.g. pulse oximetry clip, blood pressure cuff)
7. Before proceeding, ensure the animal has reached an appropriate depth of anaesthesia/chemical restraint:
   i. Light plane of anaesthesia – for minor procedures only (e.g. non-surgical/non-invasive, minimal intervention requiring immobilization only):
      - Muscle tone must be loose/weak

- Skin pinch reflex should be absent, i.e. superficial pain should be absent (see table 1)

If performing non-invasive imaging (e.g. MRI), whereby only chemical restraint is required, alternative anaesthetics or doses, than that described in Appendix A, would be required. Contact UQBR veterinary services for support.

ii. Deep plane of anaesthesia – for major procedures (e.g. surgical/invasive, major intervention):
   - Muscle tone must be loose/weak
   - Skin pinch reflex must be absent, i.e. superficial pain is absent
   - Toe pinch reflex must be absent, i.e. deep pain is absent (see table 1)

The anaesthetics at the doses described in Appendix A should cause general anaesthesia, as compared to sedation, and result in a deep plane of anaesthesia.

8. Perform the approved procedure (**).
9. Once the procedure is completed, place rodent into an individual cage within the pre-warmed recovery area (recovering animals should not be placed with non-anaesthetised animals - refer to UQBR Guideline 2 Rodent Heating Procedures).
10. Continuously monitor the rodent until it has recovered its reflexes, is normally responsive to external stimuli, and is able to ambulate, eat, and drink and toilet normally.
11. Heated ventilated chambers (similar to a humidicrib) may be used to support the animal over 12-24hours post procedure.

VI. REFERENCE INFORMATION

Table 1 | Reflexes mentioned in the above procedure, their method of assessment, and significance in anaesthetic monitoring.

Reflex	Method of assessment	Significance
Righting reflex	The animal is gently rolled onto its back. The righting reflex is lost when the animal is unable to regain an upright posture (standing or lying down).	Loss of the righting reflex (LORR) is correlated with a loss of consciousness.
Skin pinch reflex (panniculus reflex)	The loose skin over the animal's dorsal surface is pinched. This reflex is lost when the animal does not visibly respond (e.g. by flinching).	Loss of this response is loosely correlated with loss of superficial pain.
Toe pinch reflex (pedal withdrawal reflex)	One of the hind limbs is gently extended, and then the footpad is firmly pinched. The toe pinch reflex is lost when the animal does not respond by withdrawing the extended limb.	Loss of this reflex is correlated with loss of deep pain.

VII. BIBLIOGRAPHY

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VIII. Appendices

Appendix A | Dose Calculation Chart, deep plane of general anaesthesia in mice and rats. (**)

- The following table describes **high doses** of anaesthetics. It is recommended that initially 80% of the total dose is administered, then (after ~15 minutes) the remainder is administered, only if required (to "deepen" the anaesthetic)
- Atipamezole (at 1mg/kg) should be administered to "reverse" the effects of xylazine (>30min post xylazine/ketamine injection). This will result in a quicker recovery time and reduced anaesthetic risks
- Zoletil® individually (at 80mg/kg), will provide chemical restraint only – it is not appropriate for surgery (without xylazine in combination)
- Other injectable anaesthetic drugs may be acceptable options for your model and strain differences can significantly impact dose requirements – these should be discussed with a UQBR veterinarian prior to their use.

MOUSE - Ketamine & Xylazine (100mg/kg & 10mg/kg, respectively)
This dose permits ~30 min of "deep" surgical anaesthesia (and 1-2hrs sleep time)

Formulating stock solution (total 5mL)			Volume of stock solution to inject	
Individual compound (original concentration)	**Volume of individual compound combined to make 5mL of stock solution**	**Drug concentration within stock solution**	**Mouse weight**	**Volume to inject**
Ketamine (100mg/mL)	0.5mL	10mg/mL	20g	200uL
Xylazine (20mg/mL)	0.25mL	1mg/mL	25g	250uL
Water for injection	4.25mL	-	30g	300uL
			35g	350uL

RAT - Ketamine & Xylazine (100mg/kg & 10mg/kg, respectively)
This dose permits ~30 min of "deep" surgical anaesthesia (and 2-4hrs sleep time)

Formulating stock solution (total 5mL)			Volume of stock solution to inject	
Individual compound (original concentration)	**Volume of individual compound combined to make 5mL of stock solution**	**Drug concentration within stock solution**	**Rat weight**	**Volume to inject**
Ketamine (100mg/mL)	2.5mL	50mg/mL	200g	400uL
Xylazine (20mg/mL)	1.25mL	5mg/mL	250g	500uL
Water for injection	1.25mL	-	300g	600uL
			350g	700uL
			400g	800uL

MOUSE - Zoletil® & Xylazine (40mg/kg & 10mg/kg, respectively).
This dose permits ~30 min of "deep" surgical anaesthesia (and 1-2hrs sleep time)

Formulating stock solution (total 5mL)			Volume of stock solution to inject	
Individual compound (original concentration)	**Volume of individual compound combined to make 5mL of stock solution**	**Drug concentration within stock solution**	**Mouse weight**	**Volume to inject**
Zoletil (100mg/mL)	0.2mL	4mg/mL	20g	200uL
Xylazine (20mg/mL)	0.25mL	1mg/mL	25g	250uL
Water for injection	4.55mL	-	30g	300uL
			35g	350uL

Appendix B | Anaesthetic monitoring record for rodent anaesthesia. This template may benefit from adjustments to suit individual, specific anaesthetic procedures.
[Mucous membrane (MM) colour should be light pink; Temperature should not drop below 35.5°C; Respiratory rate (RR) will vary (60-220/min) but rapid drops should be of concern; Arterial blood oxygen saturation (SpO2) should be >95%; Heart rate and blood pressure is not listed but should be added if equipment is available that can accurately measure these parameters]

General details	Animal details	Anaesthetics and analgesics details
Date of anaesthesia:	**Species/strain/sex:**	**Drug dose and route:** / **Drug concentration (and volume injected):** / **Time:**
AEC approval #:	**Rodent ID:**	
Procedure:	**Animal's condition:**	
Experimental details:	**Body Weight:**	
Personnel (and roles):	**Notes:**	

Duration (min) /Time	Position	Righting Reflex [s]	Muscle tone [s]	Skin pinch [s]	Toe/tail pinch [s]	MM colour	Temp (°C)	RR	SpO2	Comments [*anaesthetic start and end; †surgery start and end]

Those criteria marked with "[s]" may be assessed as: absent (-); only mildly present (+); present but dull (++); present/normal conscious response (+++); Loss of righting reflex (LORR)

Comments:

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

Appendix C | Example of a completed anaesthetic monitoring record from injectable anaesthesia (and surgical procedure) performed in mice.

General details	Animal details	Anaesthetics and analgesics details
Date of anaesthesia: 17/11/2020 **AEC approval #:** 465/21 **Procedure:** Surgery - Tumour resection (flank) **Experimental details:** $1x10^6$ RMA/S injected SC (03/11/2020) **Personnel (and roles):** TB (surgery); KS (assistant)	**Species/strain/sex:** Mouse: CBA/J (male) **Rodent ID:** #7 **Animal's condition:** Bright, alert, normally responsive **Body Weight:** 25g **Notes:** 1 of 6 tumour resections today	

Drug dose and route:	Drug concentration (and volume injected):	Time:
Ketamine 100mg/kg IP Xylazine 10mg/kg IP	10mg/mL & 1mg/mL (=250uL) 80% injected = 200uL	09:15
Atipamezole 1mg/kg SC	0.5mg/mL (=50uL)	10:00
Meloxicam 2mg/kg SC	0.5mg/mL (=100uL)	09:25 (+1 repeat dose in 24hours)

Duration (min) /Time	Position	Righting Reflex [s]	Muscle tone [s]	Skin pinch [s]	Toe/tail pinch [s]	MM colour	Temp (°C)	RR	SpO2	Comments [***anaesthetic start and recovery/end; †††surgery start and end]
0 / 09:15	ambulatory (a)	+++	+++	+++	+++	Pink (p)	conscious (c)	(c)	(c)	***, ket/zyl (80 & 8mg/kg = 200uL)
10 / 09:25	Lateral	-	-	+	+	p	38.8			09:27 LORR; oxygen (via nose cone), meloxicam (2mg/kg)
20 / 09:35	Lateral	-	-	-	-	p		120	95%	surgical skin prep
30 / 09:45	Lateral	-	-	-	-	p		108	93%	†††
45 / 10:00	Lateral	-	-	-	-	p		112	96%	†††, atipamezole (1mg/kg)
60 / 10:15	(a)	+++	+++	+++	+++	p	conscious (c)	(c)	(c)	***, returned to recovery cage
75 / 10:30	(a)	conscious (c)	(c)	(c)	(c)	p	(c)	(c)	(c)	Quiet, normally responsive
135 / 11:30	(a)	(c)	(c)	(c)	(c)	p	(c)	(c)	(c)	Bright, alert, normally responsive
195 / 12:30	(a)	(c)	(c)	(c)	(c)	p	(c)	(c)	(c)	Bright, alert, normally responsive

Those criteria marked with "[s]" may be assessed as: absent (-); only mildly present (+); present but dull (++); present/normal conscious response (+++); Loss of righting reflex (LORR)

Comments:	Procedure performed routinely. Repeat dose: 2mg/kg meloxicam (100uL SC) to be administered at 09:00, 18/11/2020 by TB.

Conditions:

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