Curtin University Standard Operating Procedure

GENERAL ANAESTHESIA FOR RODENTS IN BUILDING 300

Number: TEC 01
Version: 1.2
Date: 01/04/2012

Aims / Objectives: To provide current recommendations for general anaesthesia for rodents used in Building 300, Curtin University. Any use of anaesthetics on mice or rats must have the approval of the Curtin University Animal Ethics Committee.

Definitions:

General Anaesthesia:
- Involves the loss of consciousness, loss of sensation, and muscle relaxation. Depending on the procedure to be performed, the level of anaesthesia can vary. It does not necessarily mean that a loss of consciousness equates with analgesia, with noxious stimuli still being able to be transmitted to, and processed by, the central nervous system.
- Can be provided by inhalational gases or vapours, or by an injection of an anaesthetic drug or mixture of agents.

Premedication: depending on the procedure to be done, and the anaesthetic agents to be used, premedication is used in some situations to provide analgesia during the procedure; reduce stress prior to the procedure; and to reduce the side effects of the anaesthetic agent used.

Fasting: is necessary in some animals prior to anaesthesia to prevent emesis (vomiting) during the procedure, however is not generally necessary in rodents as they don’t normally vomit during the induction of anaesthesia.

Intraperitoneal (IP) – is the injection of a substance into the abdominal cavity of the animal to be absorbed rapidly by the peritoneal lining into the animal’s blood stream.

Subcutaneous (SC) – is the injection of a substance under the skin of the animal into the subcutaneous tissue and to be absorbed into the animal’s blood stream.

Xylazine – an alpha2-agonist analgesic drug
Ketamine- rapid-acting, non-narcotic, non-barbiturate agent used as a dissociative anaesthetic, and commonly used in combination with other drugs to produce general anaesthesia. It is an S8 drug which is strictly regulated.

Scheduled Drugs - Use of schedule 4-8 drugs

a. Anaesthetic drugs are supplied by prescription under a veterinary licence or research permit.

b. Storage - Schedule 8 drugs, which includes the opiates and ketamine, should be stored in a locked safe with restricted access.

c. Dispensing records for these drugs are made in a bound book. For therapeutic use (including anaesthesia) they should only be issued under the direction of a veterinarian.

Procedures:
The recommended protocols and anaesthetic dosages are listed below:

Inhalational Anaesthetics:

It is recommended that isoflurane is used if possible, and the surgery room and anaesthetic equipment in Building 300 is set up correctly. This is a non-flammable liquid which is administered by vaporising it into a usable gas using a precision vaporiser. For induction, the mouse or rat is placed carefully in the anaesthetic chamber, and a mixture of oxygen (2-3L/min) and isoflurane (4%) is pumped into the chamber from the vaporiser. The animal is anaesthetised within 1-2 minutes. The animal is then removed from the chamber, and if anaesthesia needs to be maintained, the mouse or rat can be maintained using a face or nose mask with isoflurane at 1-2.5 % (NHMRC, 2008). The oxygen should be maintained at 0.5-1L/minute.

It is relatively simple to administer with the correct equipment, the depth of anaesthesia can be easily controlled, and allows a rapid recovery from the anaesthesia when the isoflurane vaporiser is turned off.

It is important that there is good ventilation and scavenging equipment (present in Building 300) whenever isoflurane is used.

Isoflurane provides no residual pain relief, so if the surgery is a recovery procedure, pain relief should be provided prior to the commencement of surgery (20 -40 minutes is optimal). This can be discussed with the veterinarian.

Injectable Anaesthetics:

1) **Xylazine / Ketamine**: this is the most common injectable combination of drugs used to anaesthetise animals and is given as an IP injection. It provides 20-30 minutes of anaesthesia to the animal, and can be topped up with either repeat injections (of either the mixture or ketamine alone, though the latter may not provide full surgical
anaesthesia), or gaseous isoflurane anaesthesia (recommended), especially for procedures lasting more than 30 minutes.

<table>
<thead>
<tr>
<th>Xylazine/ Ketamine</th>
<th>Xylazine</th>
<th>Ketamine</th>
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<tbody>
<tr>
<td>Mouse</td>
<td>10mg/kg IP</td>
<td>80-100mg/kg IP</td>
</tr>
<tr>
<td>Rat</td>
<td>10mg/kg IP</td>
<td>75-100mg /kg IP</td>
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(NHMRC, 2008)

The xylazine can be reversed with the use of Atipamezole once the procedure is finished:

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<tbody>
<tr>
<td>Mouse</td>
<td>1mg/kg SC or IP</td>
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<tr>
<td>Rat</td>
<td>1mg/kg SC or IP</td>
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(NHMRC, 2008)

2) Medetomidine (Domitor®) / Ketamine – Medetomidine (an alpha2-agonist similar to xylazine) is replacing the use of xylazine in many animals due to its safety margin, and when used with ketamine, produces a deeper anaesthesia, and it is more reliably antagonised by atipamezole. It appears to have fewer side effects than the xylazine.

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<thead>
<tr>
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<tbody>
<tr>
<td>Mouse</td>
<td>1mg/kg IP</td>
<td>75mg/kg IP</td>
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<tr>
<td>Rat</td>
<td>0.5mg/kg IP</td>
<td>75mg/kg IP</td>
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(NHMRC, 2008)

Reversal of Medetomidine with Atipamazole

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(NHMRC, 2008)

Barbiturates:
These drugs are not recommended for anaesthesia, though they have been regularly used in the past. They can be used for non-recovery surgeries by using a diluted form of Lethobarb® to induce a state of unconsciousness before respiratory depression and death. It only has a narrow safety of margin so the dosage needs to be calculated carefully. The barbiturates are given IP and at a dose of 40-50 mg/kg (NHMRC, 2008) for both rat and mouse. This must be approved by the AEC.

ANAESTHETIC MONITORING

1. Pre anaesthetic monitoring
   a. Take note of the animals demeanour, respiratory effort and rate, body score
   b. Weight
   c. No fasting is required for mice and rats prior to induction

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2. **Intraprocedural monitoring**

   a. Animals should be continually monitored during anaesthesia. It is desirable to have an assistant for this process if a surgical procedure is being carried out to maintain aseptic technique.

   b. Parameters to be monitored:
      i) Respiratory rate and character. Fast shallow respiration indicates lightening of the anaesthetic plane; slow deep breaths indicate deepening.
      ii) Reflexes. Absence of a pedal withdrawal reflex is the most reliable indication of attainment of a surgical plane of anaesthesia in rodents.
      iii) No response to external stimuli.
      iv) Body temperature - since hypothermia is of concern in small animals.

   c. Hypothermia can prolong recovery. Rodents should be provided with additional heat at all times when anaesthetised and in the immediate recovery period. Warm air blankets, heating pads and heating lamps can be used for this purpose. Careful placement of heat sources and monitoring is needed to prevent thermal burns.

3. **Post-procedural monitoring**

   a. The animal should be observed continuously until the righting reflex has returned by the researcher or an assistant. Animals should be returned to a warm, draft-free cage that is placed on a warming pad or under a heat lamp. Animals at different stages of recovery should not be put together as more awake animals are likely to trample others. However return to social housing as soon as possible.

   b. If surgery has been performed the bedding substrate should not be able to stick to the wound e.g. use paper rather than wood shavings as bedding.

   c. Ensure that the animal is able to reach water and food sources. If not then place these on the cage floor or consider administering fluids to prevent dehydration. Hydrating gels are also commercially available and are very useful in the post-operative period. Dehydration can be monitored by persistence of a skin tent.

   d. Monitor the animals’ basic biological functions (food/water intake, bodyweight, urination) as well as any clinical signs of distress daily for at least the first week following surgery. If surgery has been performed then an analgesic plan should have been devised and this should be followed and recorded. Post-op care sheets are very useful for recording such information and should be used wherever possible (see Post Procedural Form 8 and Health Monitoring Cards Form 1).
Please consult with the Animal Facility Staff if the above mentioned anaesthetics are not suitable for your work.

Additional resources:
2) The University of British Columbia website: http://www.animalcare.ubc.ca/anesthesia_analgesia.html

Author: Dr Tara Pike

Date of Approval: 01/04/2012

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<tr>
<td>12/06/2014</td>
<td>Dr Tara Pike</td>
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<tr>
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