

Experimental Techniques and Anaesthesia in the Rat and Mouse

**ANZCCART
Facts Sheet**

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Introduction

The use of animals in basic biological research and in research applied to specific purposes has made major contributions to the welfare of man and other animals in the treatment and prevention of disease. However, progress of experiments and the quality of life of experimental animals is closely related to the ability and training of research personnel and animal care staff handling the animals. A relatively simple procedure, such as blood sampling, can become a traumatic experience for the animal if performed roughly or incompetently by an unskilled or unsympathetic person. Familiar staff and competent handling can do much to reduce the fear and distress that many animals may otherwise feel. The following notes have been collated by experienced animal technicians to provide some guidance in the use of a number of commonly performed techniques.

Handling and restraint

It is very important both for the safety and comfort of the animal and of its handler that the animal is properly restrained before any manipulations are attempted.

Mice can be picked up by the base or middle third of the tail and placed on the cage lid. They can then be restrained by grasping the loose skin behind the ears with the thumb and fore-finger, while keeping some tension on the tail. If the skin is grasped too loosely or over the shoulders rather than behind the ears, the mouse may be able to turn its head and bite. Once the mouse has been 'scruffed' it can then be picked up and its tail held between the fourth and little fingers. It can easily be held securely, allowing administration of injections or palpation of the abdomen.

Rats should not as a rule be handled with gloves or forceps. They learn quickly and will soon become accustomed to the investigator's hand. They should be approached from behind and grasped firmly around the neck by the thumb and fore-finger, with one fore-leg encircled. The other hand may be used to support and restrain the hind legs.

A number of mechanical devices can be used to restrain rats and mice and are usually used for giving intravenous (iv) injections via the tail vein. The animal is restrained either in a

cylinder with a plunger which can be adjusted to the size of the animal, or a cone shaped device, which restrains the body but allows the tail to be free.

Chemical restraint in the mouse and rat

Inhalant anaesthetics

One to two ml of an inhalant anaesthetic (halothane, methoxyfluorane or isofluorane) is placed on a cotton wool pad in a bell jar or screw top glass jar. This method of anaesthesia is often used for short term procedures where the animal needs to be anaesthetized for only a few minutes. When the liquid has been placed on the pad in the jar, the lid is replaced, allowing the vapour to fill the jar. The mouse or rat is then placed in the jar and removed when fully anaesthetized. Should the animal need to be maintained, the application of a nose cone containing a small quantity of the anaesthetic can be carried out at regular intervals. It is always desirable to have a grid between the animal and the soaked cotton pad, so that the animal does not physically contact the anaesthetic. This method of anaesthesia should always be carried out in a fume hood and the container should always allow easy viewing of the animal.

The inhalant anaesthetic can also be delivered by an anaesthetic machine, in combination with O₂ and N₂O, either into a nose cone or perspex box with scavenging system incorporated. This is much safer than the open drop method if using halothane or isofluorane, as the animals can become deeply anaesthetized very quickly and the use of a calibrated machine gives far greater control of anaesthetic depth.

Injectable anaesthetics (see table 1)

Injection techniques - rats

Untrained personnel should always have an assistant when attempting any techniques, that is, one person restraining and the other applying the technique. Only fully trained personnel should attempt to restrain an animal and give an injection without assistance.

Intramuscular injections (i/m)

Anaesthetic - not required. Needle size and gauge - 25mm/23-27 gauge.

Thigh muscles that can easily be injected are the tensor fascia lata, biceps femoris, vastus lateralis and semitendinosus. Any injection into these sites risks damage to the sciatic nerve. One operator should firmly place an index finger and thumb under the chin (mandibles) of the rat, carefully lifting and supporting the body while the free hand grasps the base of the tail or pelvis. Once the animal is securely restrained it can be presented to the other investigator to administer the injection. The person administering the injection can firmly grasp the paw of either leg and gently stretch the limb so that most of the muscles are extended. The investigator should insert a short, small gauge needle on a parallel plain to the femur into the posterior muscles, while aspirating the plunger to ensure that blood vessels have not been breached. Only small volumes can be injected intramuscularly (max. 0.05 ml).

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Intra-peritoneal injection (i/p)

Anaesthetic - not required. Needle size gauge - 25mm/23-27 gauge.

Rats are restrained as for the i/m injection. Two major methods of administration are practised. First, the rat is slightly tilted so that its head is facing towards the floor. This allows the abdominal organs to move toward the thoracic cavity enabling the second operator to insert the needle laterally to the midline, (imagine a quadrant drawn onto the rat's abdomen, and injecting into the lower squares), thereby avoiding major organs. The second method is to line the needle up with an outstretched leg as the needle is inserted into the peritoneal cavity. This technique also avoids major organs.

Intradermal injection (i/d)

Anaesthetic - is required. Needle size and gauge - 25mm/26-27 gauge.

Rats ideally should be anaesthetized to perform this technique, as the needle insertion requires the animal to be completely still. Only small amounts of fluid should be injected (up to 100µl). Once the animal is anaesthetized the back can be shaved. This is the usual site to administer an i/d injection. The closer the shaving, the easier it is to apply this technique. A small gauge needle is inserted into the dermal layers. If the bevel is facing the operator it can be seen through the skin after it is inserted. The needle should not be inserted past the end of the bevel. A small bulge will appear at the site if the injection is successful.

Intravenous injection (i/v)

Anaesthetic - not required. Needle size and gauge - 25mm/26-27 gauge.

Intravenous injection and collection from the rat tail is one of the most common techniques. Various methods have been adopted by investigators, based upon convenience, skill and requirements. Lateral tail vein incision, where a vacuum tube is placed over the tail, has been replaced by the use of needles and syringes. Other intravenous collection and injection techniques include cannulation or needle insertion into the femoral and/or jugular veins and needle insertion into the sublingual or the saphenous vein. The lateral tail vein bleed or injection is the most commonly used technique. The rat should be placed in a clean container with no food or water on the lid. A heat lamp should be placed approximately 10 to 20 cm from the cage lid to warm the animal, so that the tail vessel will dilate.

Rats should not be allowed to become too hot and must be continually monitored. Once the desired heat is reached the rat must be quickly removed and restrained for the needle insertion. Commercial restrainers are available although a quick, cheap and more comfortable technique for the rat is to roll it into a medium sized towel (The Duffy Rat Roll). The rat is placed into a blind fold of the towel, while the rest of the towel is rolled over and under the rat. This restraining technique can also be used when performing i/m injections or taking blood pressure

measurements in rats. Once the rat is firmly restrained, the needle can be inserted, bevel up, using a 1, 2, or 2.5ml syringe. The needle must be inserted at a shallow angle. Once in the vessel, a small amount of blood will appear in the syringe. This blood must appear before injecting, as it indicates the needle's position and ensures the material is injected in the vein. A welt will appear around the vein if the needle is not positioned correctly. If blood samples are required, slowly withdraw the plunger until the required volume is collected. Applying excessive force on the plunger will only collapse the vessel.

Subcutaneous injection (s/c)

Anaesthetic - not required. Needle size and gauge - 13 to 25mm/23 to 26 gauge. Volume - maximum 5ml.

One trained investigator can carry out this technique, providing the animal can be fully restrained without undue stress. Carefully remove the animal from its box or cage and gently place it on a non-slip surface. Once the rat is carefully restrained by the investigator, the needle can be inserted into the animal's flank, back of the shoulder or down the animal's back. The administration site will depend on the method of restraint. Working on a standard height laboratory bench, the animal can be gently pressed against the waist of the operator. One person can rest a hand on the back of the rat with the index finger and thumb positioned over the neck. A fold of skin can be gently pinched into the shape of a 'tent' while the needle is inserted parallel to the animal's body with free hand. A perpendicular needle insertion can result in the investigator jabbing a finger, or pushing the needle through the skin on either side of the fold.

With the investigator sitting at a bench, a rat can also be restrained by placing it between the forearm and waist. In the case of a right handed person the rat will be restrained by the left forearm, facing the head to the left. Using the left hand a fold of skin can be pinched and the injection given with the right hand. The use of two operators will cause less stress to the rat, as less time will be required to complete the task.

Gavage or oral dosing

Anaesthetic - not required. Needle size and gauge - 50 to 100 mm/15 to 18 gauge.

Stomach tubes or catheters are occasionally used to orally dose rats. However, the most commonly used apparatus is a metal needle with the sharp end removed - a gavage needle. Gavage needles allow the investigator to administer an accurate dose rate or volume into the oesophagus or stomach. They can be 15 to 18 gauge, with a ball of silver soldered onto the distal end, which will allow smooth entry into the mouth, through the posterior oropharynx and into the oesophagus. Needles can be straight or curved and up to 100mm long. These are available commercially, although many institutes make their own.

Table 1. Injectable anaesthetic agents

Drug	Mouse	Rat	Comments
Pentobarbitone	40-90mg/kg i/p	30-50mg/kg i/p	Anaesthetic effects can vary depending on strain, sex and age of animal. Poor analgesia, low margin of safety. 30-60 minutes duration
Ketamine plus xylazine	200mg/kg i/m plus 10mg/kg i/p	100mg/kg i/m plus 10mg/kg i/p	
Alphaxalone/alphadolone ('Saffan')	10-15mg/kg i/v	10-12mg/kg i/v	Excellent but short acting anaesthetic. Will need to top-up every 10-15 minutes.
Fentanyl/Fluanisone ('Hypnorm') plus Midazolam ('Hypnovel')	Mixture - 1 part Hypnorm 1 part Hypnovel 2 parts water		Excellent analgesia, 30-40 min. surgical anaesthetic but a long 'sleep' time. Slight respiratory depression can be reversed with buprenorphine without loss of analgesic potential.
Chloral hydrate	10ml/kg i/p	2.7 ml/kg i/p	
Tribromoethanol ('Avertin')	400mg/kg i/p	200-300mg/kg i/p	Poor analgesia and marked respiratory depression. Can cause ileus in rats.
	125mg/kg i/p	300mg/kg i/p	Good anaesthesia but solution must be freshly prepared. Post-operative death or adhesions can be sequelae.

This technique can be used by one person, although two people should be used for wild or sick animals. A single investigator can restrain a rat by firmly placing an index finger and thumb under the chin, then carefully lifting and supporting the rat's body while pressing the animal against his chest. In this position the animal is lightly restrained and reasonably comfortable. Finally, tilt the rat's head into a position so that it is facing the investigator, enabling the gavage needle to be gently inserted into the rat's mouth.

Injection techniques in the mouse

Helpful hints

- prior to administering an injection the animal must be adequately restrained, whether by the hands, a restraining device or assistance from another person,
- use your dexterous hand to inject and restrain with the other,
- keep the bevel of the needle and graduations on the syringe in line with each other and facing upwards,
- use as small a gauge needle as the substance permits to do the injection. Use a larger gauge if necessary to draw up the solution, then change to a smaller gauge,
- don't put your finger on the plunger until ready to inject.

Subcutaneous injection

Keeping the syringe in a horizontal position, insert the needle under the surface of the skin in the lower abdomen. Inject the mouse with as small a volume as possible. The dorsum of the mouse can also be used as a site of s/c injection, using loose skin over the neck.

The skin will swell at the injection site immediately, indicating that the injection is not too deep, a common error. Leave the needle under the skin for a few seconds post-injecting, then remove slowly as this will help prevent leakage. The larger the volume, the greater the likelihood of leakage.

Intra-peritoneal injection

Using the same method as for s/c injections, insert the needle under the skin surface, lift the syringe to a vertical position and push down through the abdominal wall into the peritoneal cavity and inject the animal. Watch the length of the needle and the angle at which you inject. It may be helpful to tilt the animal's head downwards so that the internal organs fall away from the injection site. It is not uncommon to inject volumes up to one ml by this route, but the amount injected will be determined by the irritancy of the solution.

Intravenous injection

Dilate the tail vein by placing the animal under a heat lamp for two to ten minutes prior to injection. A cone shaped device may be used to restrain the animals. Veins are located either side of the artery (which runs down the centre of the tail) and appear blue-red in colour when dilated. Keeping in line with the vein, insert the needle, which should be no larger than 26 gauge, into the vein about three cm from the tip of the tail. To check that the needle is in the vein, pull back on the plunger for signs of blood. If there are no signs, remove the needle and try again closer to the tail butt. Another way of checking is to depress the plunger and, if you feel any resistance, try again. However, if the plunger moves freely, continue to inject. The tail vein will collapse if it is punctured too many times.

Intravenous injection in neonates is sometimes necessary and can be performed using a 30g needle into the anterior facial vein at the level of the lateral canthus of the eye, or into the transverse sinus, which is easily identified on the surface of the cranium. Intra-peritoneal injection of neonates is often performed by inserting the needle in the skin over the thoracic cavity towards the animal's umbilicus, over the liver and then into the animal's abdominal cavity.

Intra-muscular injection

This is rarely used in the mouse, due to the very small amount of muscle mass and the fact that rates of absorption of aqueous solutions are similar to the s/c route of administration. A maximum volume of 0.05ml into the quadriceps femoris muscle group is possible.

Blood collection from the rat

Intracardiac sampling.

Anaesthetic - is required. Needle size and gauge - 25mm to 100mm/23 to 26 gauge.

As anaesthetics are involved in the application of this procedure, only experienced investigators should attempt it. Although the tail veins are in a more convenient location for access in the rat, it is difficult to obtain large volumes of blood from the tail. Intracardiac bleeds allow the investigator to obtain large volumes with each application on a fortnightly basis. The number of serial bleeds depends on the previous volumes removed, number of anaesthetics administered and the general well-being of the rat. Maximum bleed should not exceed 1% of the rat's body weight. An experienced investigator will obtain the required blood volume within one minute, once the rat is anaesthetized.

Placing the animal on a warm or protected surface on its back, the investigator can, using the index finger and thumb, locate the beating heart through the left side of the chest (between the fifth and sixth ribs) and insert the appropriately sized needle through the intercostal muscles into the left ventricle of the heart. Using the thumb or index finger as a guide for the needle ensures that a straight and direct insertion is made. If the first attempt is not successful, do not partially withdraw the needle and re-insert it at a different angle. Soft tissue damage can occur, causing internal bleeding. The needle must be completely removed from the thoracic cavity and re-inserted. Left ventricular insertion provides a strong flow into the needle. Tail clipping is not recommended, as small or large volumes of blood can be obtained using a needle and syringe. Amputating the tip of the tail causes more pain than necessary when other less invasive techniques are available to the investigator.

Tail vein

As for i/v injections (see above).

*Orbital bleeding**

Orbital sinus bleeding in the rat can be carried out in the same manner as the mouse. The rat needs to be fully anaesthetized for the procedure. A pasteur pipette or a haematocrit tube can be used for the collection, depending on the volume of blood required. The blood vessels behind the eye are harder to penetrate in the rat than the mouse and so a little more pressure is necessary along with the constant rotation of the tube to rupture the blood vessels.

Blood collection from the mouse

Blood parameters can vary markedly, depending on the site of collection. Once a method has been decided, it should not be varied.

Orbital bleeding

The mouse should always be anaesthetised unless this technique is performed by an experienced technician. A microhaematocrit tube is inserted via the lateral (or medial) canthus, at an angle of about 30° into the venous plexus behind the eye with a twisting motion and the blood allowed to flow through the tube into the collection vessel. If larger amounts of blood (up to 0.5ml for a large mouse) are required, it helps if the microhaematocrit tube is coated with an anticoagulant. Bleeding usually stops when the tube is removed.

Cardiac bleeding

This is useful for obtaining large amounts of blood. The mouse must be anaesthetised and it is usually a terminal procedure. The apical beat is palpated and the needle inserted either through the ribs or via the diaphragm under the xiphoid cartilage and slightly to the left of the midline, at an angle of 20° - 30° from the sternum.

Rederivation of a colony via caesarian section and embryo transfer

This may be used to obtain mice free of endemic disease or to eliminate unwanted pathogens from the colony.

Caesarian section

The donor female should be euthanased by cervical dislocation. A small transverse incision is made in the skin on the abdomen and the skin peeled away. The mouse is then submerged in 37°C iodine for 4 minutes. Then open the abdomen and carefully

* (See elsewhere in this issue of ANZCCART News for a report on this technique)

remove the uterus, dipping it into warm formal saline before placing on a warming board. Carefully cut along the uterus spilling the neonates from within. Use a sterile linen swab to wipe their mouths briefly, before cauterizing the umbilical cord. Move the neonates to a clean area on the board and spend a few more minutes gently wiping them, which also helps to stimulate breathing. Once they are breathing and pink in colour, they are ready to be fostered. To help the foster mother accept the pups it is a good idea to put her own pups on top of the foster pups, or for the foster mother's pups to urinate on the new litter, which assists with the transfer of smell. Be careful not to confuse the litters.

Plugging

Set the animals up for mating and make a habit of checking the female early every day for the presence of a vaginal plug. The plug consists of coagulated proteins from the seminal fluid and can usually be seen by placing the female on the cage lid and lifting the hind legs off the lid by pressing gently just above the tail base to expose the vagina. The plug is a creamy yellow colour and when gently touched with a small probe or blunt forceps will feel solid. Certain strains of animal tend to have deep implantations and blunt forceps may be used to gently open the vagina for checking. The plug usually dissolves about 12 hours after mating. The day that the plug is first noticed is counted as day 0.

Progesterone is administered to pregnant mice on the 17th and 18th day after the plug to prevent the mouse delivering naturally. This is common practice for animals that are being used for caesarians, which would be performed on the 19th day. 0.2ml progesterone* is added to 5ml of sterile peanut oil and 0.1ml is then injected s/c into the scruff of the neck. Depending on the gestation period of the strain, the dates of administration of progesterone should be adjusted accordingly.

*Medroxy progesterone acetate 50mg/ml(Upjohn).

Superovulation

A large number of immature ovarian follicles are induced to maturity by an injection of Folligon, a complex glycoprotein from the serum of pregnant mares. It is marketed in a freeze dried form. Make a 5ml volume by adding the solvent provided. Withdraw 1ml of the solution, add to 7ml of MTPBS** and mix. Inject 0.2ml/mouse i/p, then 44-48 hours later, inject the animal with human chorionic gonadotrophin (APL)***, which has luteinizing hormone activity. Ovulation should occur approximately 12 hours later. To make up the APL, mix 0.2ml of the solution with 3.8ml of MTPBS. Inject 0.2ml i/p per mouse and put the female in with the fertile male studs. The females should be plugged the following morning. Use females between 3 - 5 weeks of age, but be aware that the optimum age for superovulation can vary a little between strains. A superovulated female can produce up to 50 embryos.

**mouse toxicity phosphate-buffered saline.

***APL, Ayerst Laboratories, at a dose of 10i.u. per mouse.

Pseudo-pregnant foster mothers

Successful embryo transfer depends on the quality of the host maternal environment. Mice are spontaneous ovulators and can be made pseudo-pregnant by mating with vasectomized males during oestrus. They will display the hormonal profile of a normal pregnant female. Mate the recipient females by 9.00am on the same day that the superovulated mice are plugged and observe for plugs the following morning. An excellent description and illustration of embryo transfer and vasectomy can be found in Hogan *et al.* (1986).

Euthanasia in rats and mice

Carbon dioxide inhalation is probably the most efficient and aesthetically acceptable method of euthanasia for both the mouse and rat. The method is quick and most suitable when large numbers of animals need to be killed. The gas is piped into a purpose-built chamber or a plastic bag containing the cages of the animals to be killed from a cylinder fitted with a regulator and flowmeter. Depending on the size of the chamber, allow several minutes for the carbon dioxide to fill the chamber. If bottled carbon dioxide is unavailable, dry ice can be placed in a container of water inside the chamber containing the animals.

Care must be taken to ensure that the animals cannot come in contact with the dry ice or tip over the beaker.

Halothane, methoxyfluorane or isofluorane can be used for euthanasia, either in an anaesthetic chamber where the anaesthetic is controlled by an anaesthetic machine and vaporizer, or a glass jar, where anaesthetic soaked cotton wool or gauze is placed in the bottom. When these agents are used it should be in a fume hood or with apparatus which has a scavenging mechanism, to minimise the risk of exposure to the gas by the operator.

Sodium pentobarbitone given i/p at the rate of 10 - 15 mg/100g body weight produces unconsciousness, followed rapidly by death.

Staff performing physical methods of euthanasia must be well trained. Cervical dislocation in the mouse is carried out by placing thumb and forefinger behind the skull, holding it firmly and pulling the tail in the direction away from the body, causing dislocation of the neck. This can be used in young rats up to 150g, whilst larger rats can be stunned by a sharp blow to the back of the head. However, this should only be performed by operators who have had experience in this technique and are competent. Where the animal's brain needs to be untraumatised, an alternative is decapitation by guillotine. When this method is used, sure handling and speed reduce the stress on the animal.

ANZCCART (1993) published a detailed monograph covering this topic.

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