

LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 1 – Introduction to the Laboratory Rat

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1. THE LABORATORY RAT

The common laboratory rat, *Rattus norvegicus*, is an ideal experimental animal for several reasons: abundance of literature published pertaining to them, ease of handling, high fertility rate, short gestation period, low maintenance and disease model for various human disorders and diseases.

1.1. General biology and physiological data

- Most active at night (nocturnal)
- Curious and investigative behaviour
- Poor vision, acute sense of hearing and smell
- Social animals, adult males may require separation if aggressive
- Body temperature: 37°C
- Respiratory rate: 75-115 breaths/min
- Heart rate: 260-400 beats/min
- Daily water consumption: 10-12 ml/100 g body weight
- Daily food consumption: 10 g/100 g body weight
- Oestrous cycle: 4-5 days
- Duration of oestrus: 12 hours
- Litter size: 6-12
- Gestation: 20-22 days
- Birth weight: 5 g
- Weaning age: 21 days
- Sexual maturity: 7 weeks
- Breeding duration: 12 - 16 months
- Male adult weight: 450-550 g
- Female adult weight: 250- 300 g
- Life span: 2.5-3.5 years

2.2. Body condition (BC) scoring system

Score 1: Rat is emaciated.

- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis.
- Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.



Score 2: Rat is under conditioned

- Segmentation of vertebral column prominent.
- Thin flesh cover over dorsal pelvis, little subcutaneous fat.
- Pins easily palpable.
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.



Score 3: Rat is well-conditioned

- Segmentation of vertebral column easily palpable.
- Moderate subcutaneous fat store over pelvis.
- Pins easily palpable with slight pressure.
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.



Score 4: Rat is over conditioned

- Segmentation of vertebral column palpable with slight pressure.
- Thick subcutaneous fat store over dorsal pelvis.
- Pins over pelvis palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.



Score 5: Rat is obese

- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
- Thick subcutaneous fat store over dorsal pelvis.
- Pins of pelvis not palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.



2.3. The Rat Grimace Scale (Sotocinal et al. 2011)

The rat grimace scale is a standardized behavioral coding system that demonstrates facial expressions which can be used to assess pain in the laboratory rat.



2.4. Cage density



How many animals in a standard rat cage? Combien d'animaux par cage à rat standard?



4 adults < 200g each
4 adultes < 200g chaque

OR
OU



3 adults < 300g each
3 adultes < 300g chaque

OR
OU



2 adults > 300g each
2 adultes > 300g chaque

OR
OU



♀
With litter
Avec portée



Your Veterinarian
Votre Vétérinaire

3. HANDLING AND RESTRAINT

3.1. Manual restraint

- Before opening the cage observe the animals within. Nervous or young rats can escape quickly.
- Rats will not stay on top of the wire-bar lid of the cage. Always restrain them.
- Rats should not be held by their tail as their skin is fragile and can easily strip from the underlying tissue.
- Rats should not be scruffed by the skin on the back of the neck.
- 2 methods are commonly used:

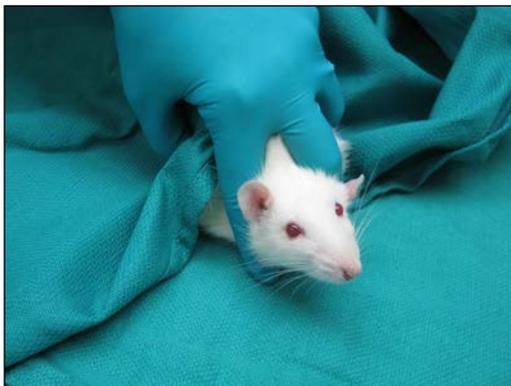
3.1.1. “V” grip

- With your non-dominant hand, slide your index and middle finger along both sides of the head as far as possible and grasp the head with your knuckles resting on the jaw bones.
- Place your thumb and remaining fingers under both forelimbs to grasp the thorax.
- If possible, support the lower body with your free hand or rest the rat on your chest (or on your legs if sitting). This is especially important for larger or gestating animals.



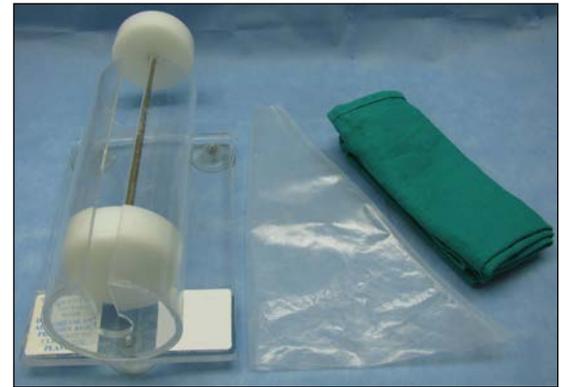
3.1.2. Towel

- Wrapping a towel around the animal will help rats feel secure and is calming, particularly if the eyes of the rats are covered.
- This method has the additional advantage of controlling the hind limbs and preventing potential scratches to the handler.



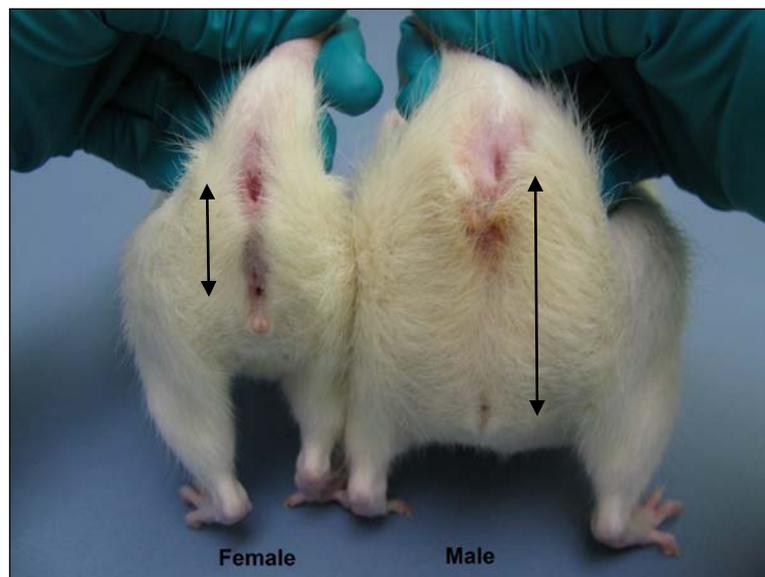
3.2. Restraint devices

- Using a restraint device can calm the rats by helping them feel secure.
- Several restraint devices are available in various sizes and materials (e.g., Plexiglas, plastic) and can be used when performing techniques such as injections or blood collection.
- The restrainer should be small enough so that the animal cannot turn around yet allow the animal to rest comfortably and breathe normally.
- Observe animals to ensure that they do not overheat and never leave an animal in a restrainer unattended.



4. SEX DETERMINATION

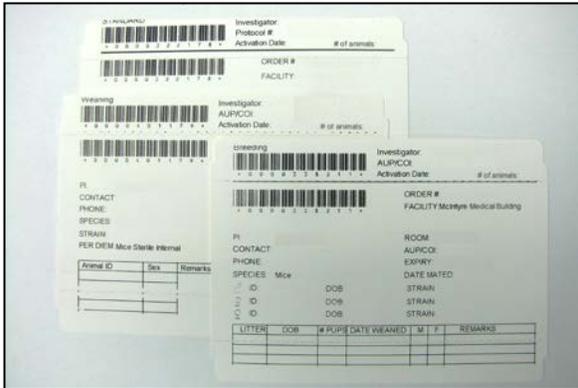
- Sexing of rats is based upon ano-genital distance
- Males have a greater distance between the anus and urogenital opening than females.
- An opposite sex comparison is advisable initially. Compare animals of similar age.
- The testicles can be retracted into the abdomen; therefore, it may be easier to sex a mature male by holding its head up vertically. The genital papilla is more prominent in males than females.



5. IDENTIFICATION

5.1. Cage cards

- All cages must have a Darwin cage card.
- Additional cage cards may be used, however, care must be taken not to cover the Darwin barcode.
- All sections of either card must be completed.



McGill			
INVESTIGATOR:	DEPARTMENT:		
CONTACT:	PHONE:		
STRAIN:	PROTOCOL:		
SOURCE:	CAGE ID:		
ANIMAL ID	SEX	DATE OF BIRTH/RECEIVED	REMARKS

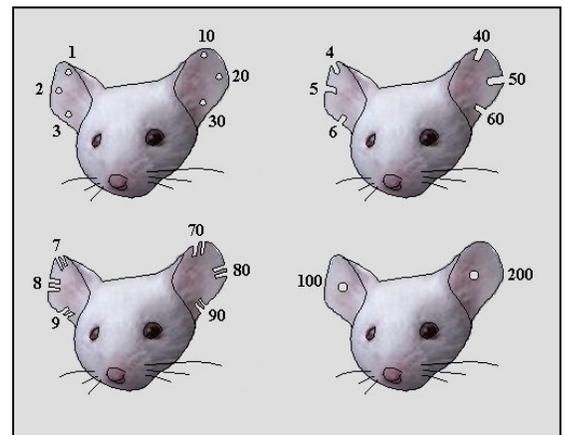
McGill		CAGE #:				
BREEDING CARD		DATE MATED:				
INVESTIGATOR:	DEPARTMENT:					
CONTACT:	PHONE:					
PROTOCOL:	SOURCE:					
STRAIN:						
♂:	♀:	♀:				
LITTER	DATE OF BIRTH	#	DATE WEANED	M	F	REMARKS

5.2. Temporary marking

- Temporary marking can be used for short term individual identification.
- Use a non-toxic, permanent marker to write numbers, bars or other distinguishable marking on the tail or the ears.
- If temporary marking is to be used for duration exceeding a week, repeat marking at least twice a week.

5.3. Ear punching/notching

- This method cannot be used on rodents under 2 weeks (14 days) of age.
- Restrain the animal securely and using an ear punch, punch a hole and/or notches in the ears following an identification chart.
- Whenever possible, use a simple code to limit the number of notches/punches made to the animal.
- Have the identification chart readily available in the animal room to allow prompt identification of individuals.
- If possible, use the excised tissue as a sample for genotyping.



5.4. Ear tag

- Use tags of appropriate size, approximately 5 mm long.
- Rinse tags in 70% alcohol before use.
- Place the tag low on the pinna (distal 1/3) so that it rests against the mouse and does not bend the ear, catch on the cage or cause the rat to hold its head in a lopsided manner.
- If the tag is placed too tight it can lead to local infection or inflammation. The animal will need to be monitored for these clinical signs and the tag removed if necessary.

5.5. Tattooing

- It is recommended to use local or general anesthesia for the procedure.
- Use an electric tattoo machine to write numbers on the tail.
- Ensure that needles are sterile and sharp.

5.6. Micro-tattooing

- It is recommended to use local or general anesthesia for the procedure.
- Use a micro-tattooer to inject tattoo ink in the toe pads and/or the ears.
- Whenever possible, use a simple code to limit the number of toes tattooed.
- Have the identification chart readily available in the animal room to allow prompt identification of individuals.

6. SAMPLING FOR GENOTYPING

6.1. Fecal pellet

- Collect fecal pellet from an individual animal using brief manual restraint or by placing it in a clean cage without bedding.
- Properly identify samples to match animal identifications.

6.2. Buccal epithelial cell

- Firmly restrain the animal by the scruff or V Grip (rats over 200g) to maintain its mouth open.
- Using the swab, vigorously scrape both inner cheeks.
- Insert cotton bud into collection tube and snip off excess shaft.
- Properly identify samples to match animal identifications.

6.3. Ear punching

- Do not use this method in rodents under 2 weeks of age.
- Restrain the animal securely.
- Using the ear punch; punch holes and/or notches in the ears following an identification chart.
- Use the excised tissue as a sample for genotyping.
- Properly identify samples to match animal identifications.

6.4. Tail snipping

- Acceptable in rats but not commonly used.
- Tail biopsy can only be performed twice over the life time of the animal and cannot exceed 5mm total.
- A maximum of 3mm of tail tip can be removed.
- Tail snipping is preferably done when pups are 14 to 17 days old.

6.4.1. Procedure for rats 14 to 21 days of age:

- General anesthesia is recommended but not required.
- Gently, but securely, restrain the rat with your hands or with the use of a restrainer.
- Swab the tail with antiseptic (e.g. chlorhexidine, alcohol).
- Snip tail with sanitized scissors or disposable scalpel.
- If you are snipping several rat tails, clean off any blood or tissues from the scissors and wipe with 70% alcohol or dip in a glass bead sterilizer for at least 30 seconds.
- Place tissue sample into the collection tube.
- Apply pressure on the tip of the tail with a clean gauze and do one of the following:
 - Apply a drop of tissue glue to the cut tip of the tail.
 - Apply a chemical cautery agent such as Kwik Stop® powder or silver nitrate stick.
 - Electric or heat cauterize the cut end of the tail.
 - Return the animal to its home cage.

6.4.2. Procedure for rats over 21 days of age:

- Requires general anesthesia and analgesia.
- Administer an analgesic such as carprofen or buprenorphine prior to the procedure and for at least 24 hours thereafter.
- Brief general anesthesia is provided with isoflurane:
 - Place the animal in the induction chamber.
 - Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.
 - Adjust the isoflurane vaporizer to 3% to 5% to achieve unconsciousness.
 - Once the animal is unconscious, adjust the flowmeter to 400 to 800mL/min and the isoflurane vaporizer to 2 to 2.5%.
- Remove the animal from the induction chamber and quickly proceed with the tail snipping as described above.
- Return the animal to its home cage once it regains consciousness.

7. EUTHANASIA

Rat can be euthanized in a variety of acceptable, effective and humane methods. Euthanasia methods can be either chemical or physical.

7.1. Adult rodents - Chemical methods

7.1.1. CO₂ asphyxiation under isoflurane anesthesia

- It is preferable to anesthetize rodents with isoflurane prior to exposure to CO₂ to minimize pain and distress.
- In order to minimize stress animals should be euthanized in their home cage according to cage density or one litter per cage (do not pool rats from different cages).
- Choose an adequately sized induction chamber and connect it to the euthanasia station.
- Place the animal cage, with filter top removed, in the induction chamber.
- Open the oxygen tank and set the flowmeter to maximum flow rate.
- Set the isoflurane vaporizer to 5%.
- Observe the animals closely. Soon after loss of consciousness (when the breath rate is still relatively high) close the vaporizer and the oxygen tank.
- While the animals are still unconscious, promptly open the CO₂ tank and set the flowmeter to maximum flow rate.
- Maintain the CO₂ flow until the animal has stopped breathing. Note that the time required for euthanasia can be several minutes.
- Close the CO₂ flow meter and the valve on the CO₂ tank.
- Leave the animals in contact with CO₂ for an additional 2 minutes, minimum.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change or opacity in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

7.1.2. CO₂ asphyxiation

- CO₂ alone should not be used where other methods are practical for the experiment and the species.
- In order to minimize stress animals should be euthanized in their home cage (do not pool rats from different cages).
- Place the appropriate sized lid on the animal cage with grid removed.
- Connect the regulator hose to lid fitting.
- Do not pre-charge the chamber.
- Plug in the heater unit if necessary (e.g., if euthanizing many cages)
- Open the CO₂ tank valve.
- Set the regulator to the appropriate setting:
 - Standard rat cage (12" x 9" x6"): 5.25 LPM

- Cages of different dimensions: a gradual-fill rate of less than 30% and greater than 20% of the chamber volume per minute should be used.
- Once the animals have become unconscious, the flow rate can be increased to minimize the time of death. Please note that the time required for euthanasia can be several minutes.
- Maintain the CO₂ flow until the animal has stopped breathing.
- Close the valve on the tank.
- Leave the animals in contact with CO₂ for an additional 2 minutes, minimum.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane colour, no response to toe pinch, colour change or opacity in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

7.1.3. Barbiturate or injectable anesthetic overdose

- Inject three times the anesthetic dose intravenously or intraperitoneally.
- Animals should be placed in cages in a quiet area to minimize excitement and trauma until euthanasia is complete.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane colour, no response to toe pinch, colour change or opacity in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

7.1.4. Overdose of inhalant anesthetic

- Anesthetic chambers should not be overloaded and need to be kept clean to minimize odors that might distress animals subsequently euthanized.
- The animal can be placed in a closed receptacle (bell jar) containing cotton or gauze soaked with an appropriate amount of the anesthetic. Because the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors. Procedures should be conducted in a chemical fume hood to prevent inhalation of the anesthetic by personnel.
- The anesthetic can also be introduced at a high concentration from a vaporizer of an anesthetic machine connected to an adequate scavenging system, air filter or type II B2 BSC.
- Sufficient air or O₂ must be provided during the induction period to prevent hypoxemia. In the case of small rodents placed in a large container, there will be sufficient O₂ in the chamber to prevent hypoxemia.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane colour, no response to toe pinch, colour change or opacity in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax, is required on your animals before disposal to ensure that they have been correctly euthanized.

7.2. Adult rodents - Physical methods

Physical methods of euthanasia are also an appropriate means to assure death after euthanasia with isoflurane, CO₂ or injectable anesthetics used as euthanasia agents. Personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique performed.

Anesthesia or sedation is necessary prior to physical methods of euthanasia, unless otherwise described in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).

7.2.1. Cervical dislocation

- Cervical dislocation, as a primary or secondary method of euthanasia, is not to be used on rats weighing over 200g.
- The thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a narrow, blunt instrument such as the dull edge of a scissor blade, acrylic ruler or cage card holder is pressed at the base of the skull.
- With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull.

7.2.2. Pneumothorax

- Cut through the skin and muscle of the abdomen just below (caudal to) the thorax.
- Lacerate the diaphragm with a sharp pair of scissors.

Note: If the animal is deeply anesthetized, the heart could be removed to accelerate the process and insure death.

7.2.3. Decapitation

- Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available.
- The use of plastic cones to restrain animals is recommended as it reduces distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.
- The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.

7.3. Neonatal rodents

Rodents over 10 days old can be euthanized by the same procedures as adult rodents.

Rodents under 10 days old must be euthanized by one of the following methods:

7.3.1. CO₂ asphyxiation under isoflurane anesthesia:

- Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations and exposure to CO₂, therefore, alternative methods are recommended.
- Isoflurane/CO₂ may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades).
- Keeping neonates warm during isoflurane/CO₂ exposure may decrease the time to death.

7.3.2. CO₂ asphyxiation

- Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂, therefore, alternative methods are recommended.
- CO₂ may be used for narcosis of neonatal animals but it must be followed by another method of euthanasia (e.g. decapitation using sharp blades).
- Keeping neonates warm during CO₂ exposure may decrease the time to death.

7.3.3. Barbiturate overdose

- Inject 3 times the anesthetic dose IP.
- Decapitation (using sharp blades) is recommended on your animals before disposal to ensure that they have been correctly euthanized.

7.3.4. Overdose of inhalant anesthetic followed by decapitation

- Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations, therefore, alternative methods are recommended.
- Inhalant anesthetics may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades).

7.3.5. Decapitation

- Guillotines are not commercially available for neonatal rodents, but sharp blades (e.g. scissors) can be used for this purpose.
- The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.

7.4. Gestating rodents

Gestating rodents with foetuses under 17 days old can be euthanized by the same procedures as adult rodents.

Gestating rodents with foetuses over 17 days must be euthanized by one of the following methods:

7.4.1. CO₂ asphyxiation under isoflurane anesthesia:

- CO₂ asphyxiation under isoflurane anesthesia of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.

7.4.2. CO₂ asphyxiation

- CO₂ asphyxiation under isoflurane anesthesia of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.

7.4.3. Overdose of injectable anesthetics to the mother.

RODENT EUTHANASIA

METHODS OF EUTHANASIA	CHEMICAL				PHYSICAL		
	CO ₂ ASPHYXIATION UNDER ISOFLURANE ANESTHESIA	CO ₂ ASPHYXIATION	BARBITURATE OR INJECTABLE ANESTHETIC OVERDOSE	INHALANT ANESTHETIC OVERDOSE	CERVICAL DISLOCATION	PNEUMOTHORAX	DECAPITATION
 <ul style="list-style-type: none"> • Adult rodent • Gestating rodent (under 17 days gestation) 	YES	YES	YES	YES	YES Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC	YES Only after a chemical method of euthanasia or under anesthesia	YES Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC
 <ul style="list-style-type: none"> • Gestating rodent (over 17 days gestation) 	YES*	YES*	YES	YES*	YES* Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC	YES* Only after a chemical method of euthanasia or under anesthesia	YES* Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC
* Decapitation of pups required after euthanasia of the mother. If barbiturate or injectable anesthetic overdose is used to euthanize the mother, decapitation is not required.							
 <ul style="list-style-type: none"> • Pups less than 10 days old 	Only as Narcosis Followed by another physical method of euthanasia	Only as Narcosis Followed by another physical method of euthanasia	YES	Only as Narcosis Followed by another physical method of euthanasia	NO	NO	YES

8. BLOOD COLLECTION BY INTRACARDIAC PUNCTURE

- Terminal procedure.
- This procedure can only be done under anesthesia or less than a minute after euthanasia.

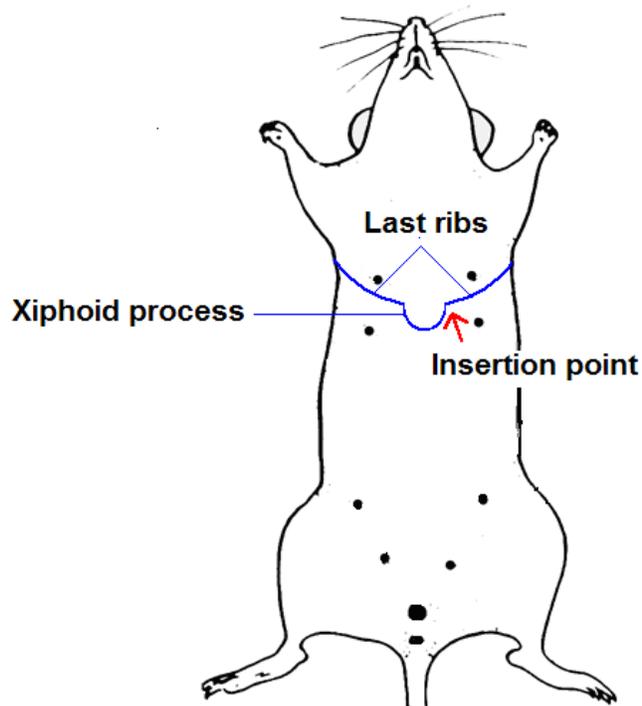
8.1. Procedure

- Prepare a syringe between 3cc and 10cc with a 20G 1½" needle.
- Place the rat in dorsal recumbency.
- Palpate the xiphoid process between the last two ribs at the tip of the sternum.
- Insert the tip of the needle between the left side of the xiphoid process and the last rib.
- Once you puncture the skin, gently pull back on the plunger to create a minimal amount of negative pressure within the syringe and maintain it.
- Penetrate the thoracic cavity slowly while directing your needle toward the heart at an angle of approximately 40-45 degrees.

Note: The heart is slightly left of the midline.

- When a small quantity of blood flows into the hub of the needle, stabilize your needle and continue to pull back on the plunger slowly. The blood should flow into the syringe at a steady rate.

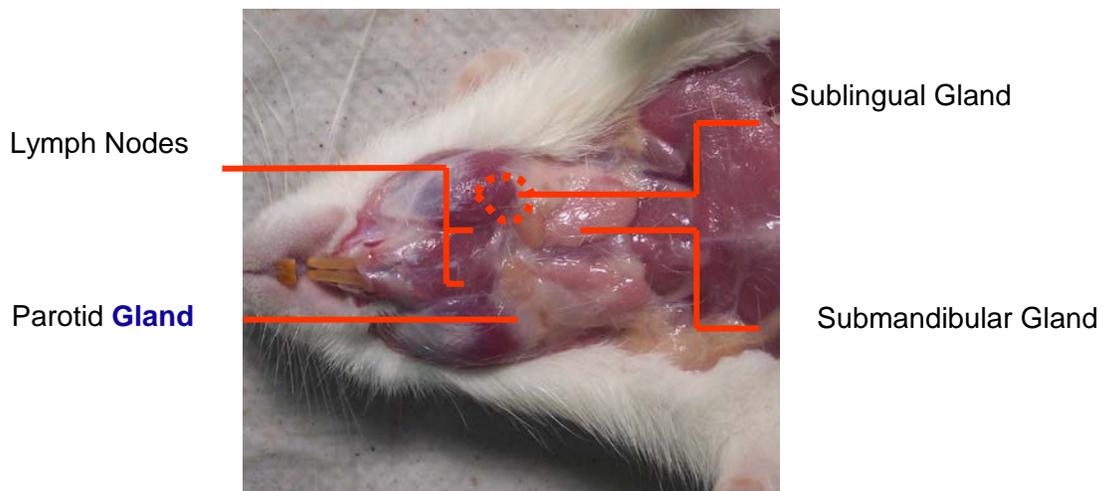
Note: If the blood flow stops, you change the angle of the needle slightly, rotate it or make very small movements to alter the needle placement.



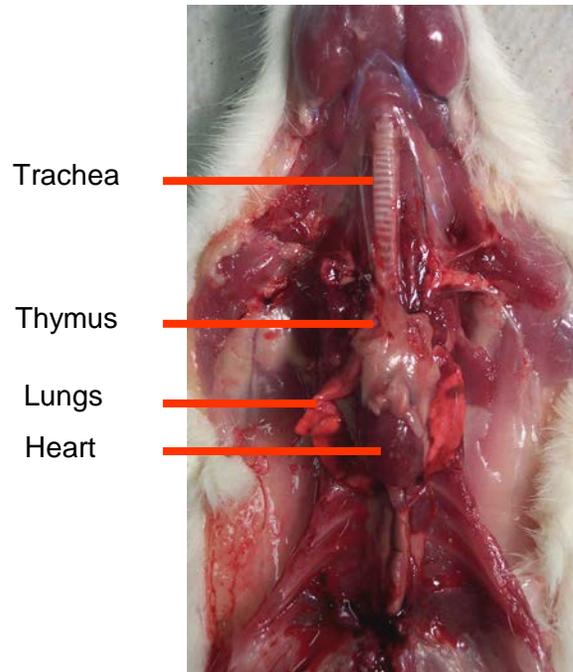
9. NECROPSY



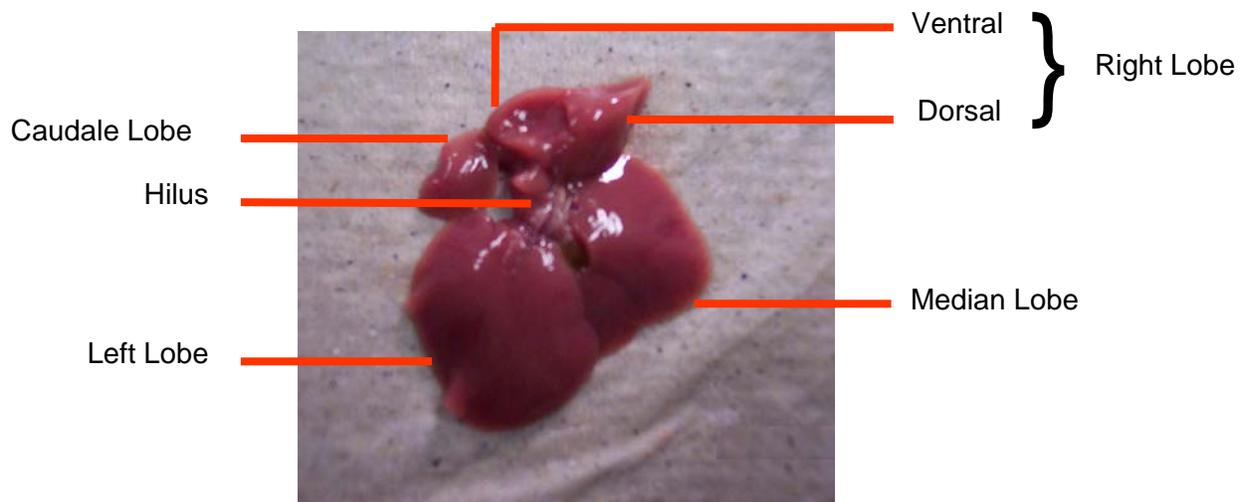
9.1. Salivary glands



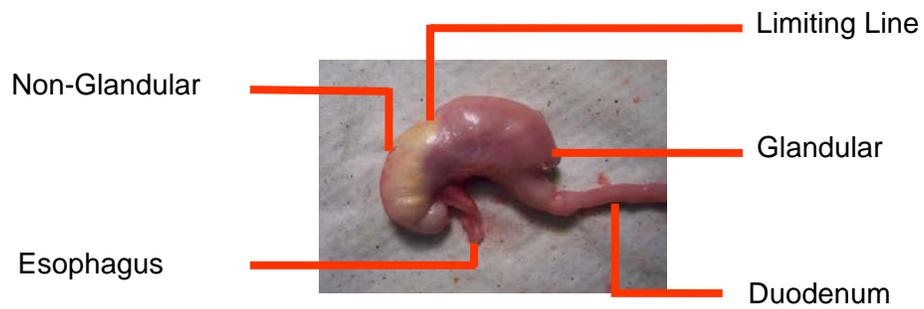
9.2. Trachea, thymus, lungs and heart



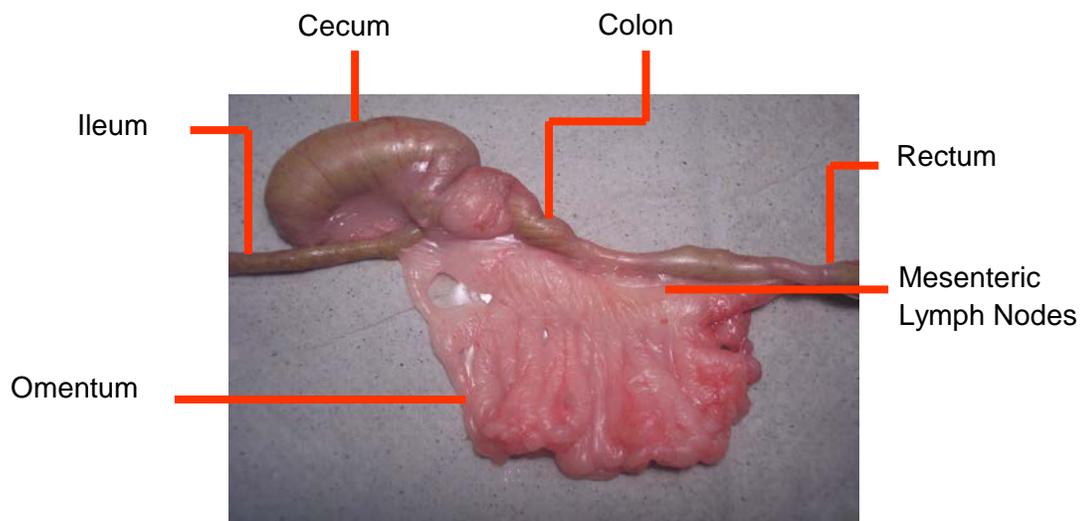
9.3. Liver



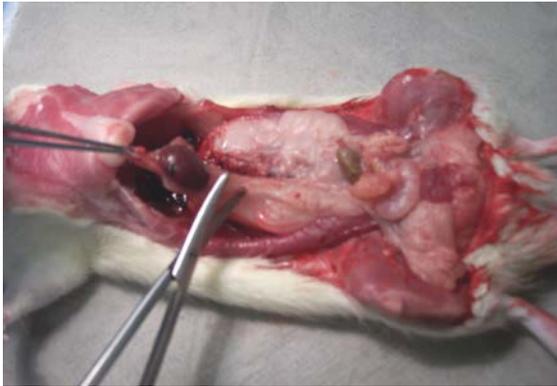
9.4. Stomach



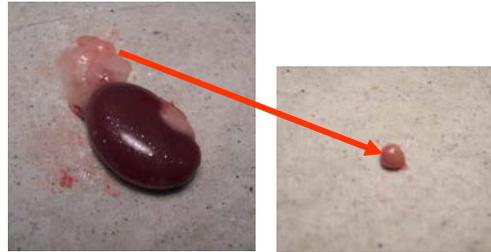
9.5. Cecum, mesenteric lymph nodes, colon and rectum



9.6. Kidney and adrenal gland

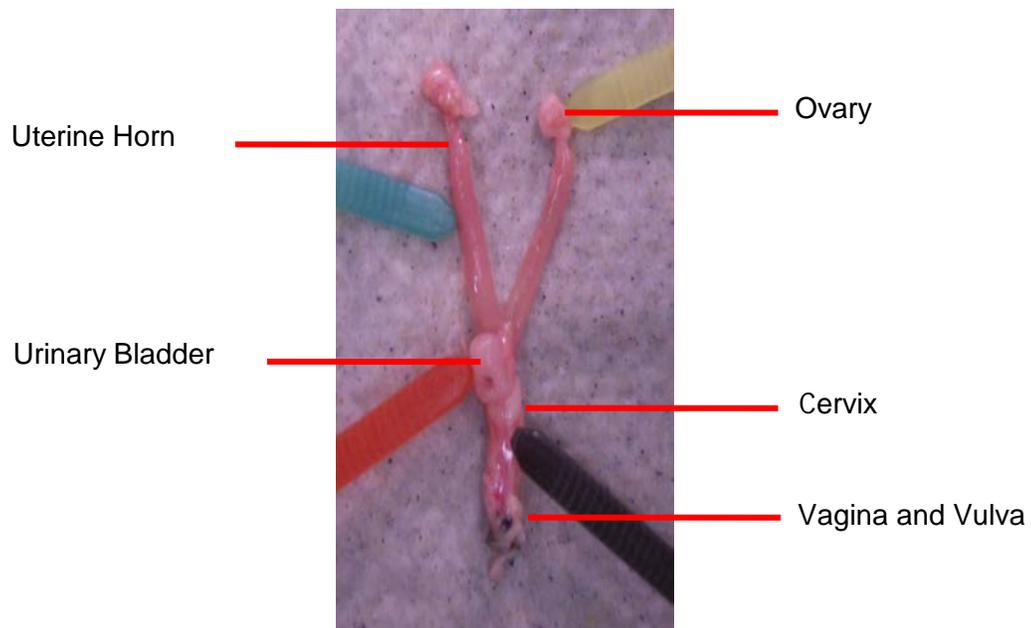


It is normal to find the right kidney displaced a little more cranially than the left in both rats and mice.

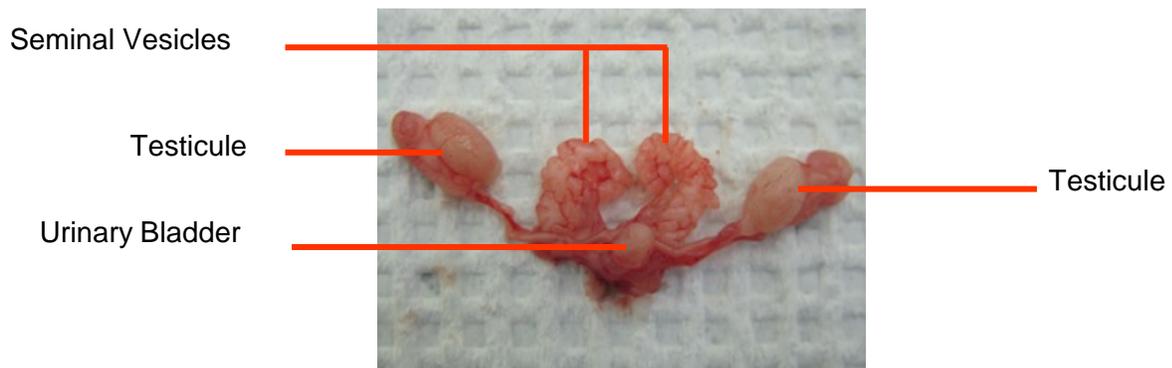
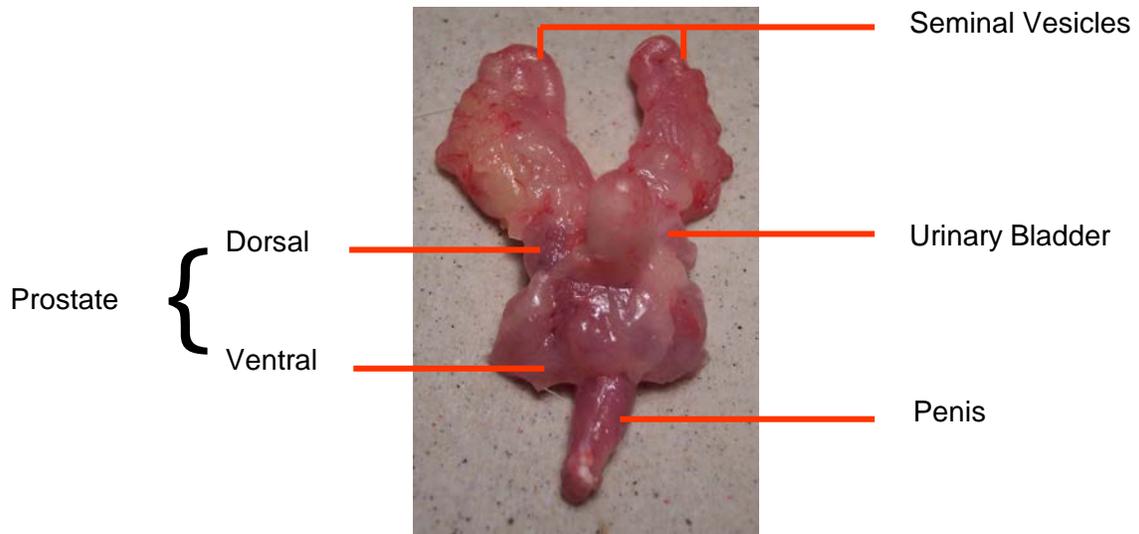


The adrenal gland may be found within the adipose tissue above the kidney, so be careful when dissecting the fat away.

9.7. The female reproductive system



9.8. The male reproductive system



Necropsy photos courtesy of Animal Health Technicians from the Douglas Hospital animal facility and from the Comparative Medicine Animal Resources Centre.

10. REFERENCES

10.1. Comparative Medicine & Animal Resources Centre

CMARC website	www.mcgill.ca/cmARC
Veterinary Care	aht.arc@mcgill.ca
Technical Services, Equipment rental (Anesthetic machines)	rts.arc@mcgill.ca
Imports, Transfers and Quarantine	import.cmARC@mcgill.ca
Imaging Services	imaging.cmARC@mcgill.ca
Irradiator Services	irradiator.cmARC@mcgill.ca
Workshop and Training	workshop.cmARC@mcgill.ca
Polyclonal Antibody Production	antibodyproduction.cmARC@mcgill.ca
Materials and drug sales	drss@mcgill.ca
Comparative Pathology	comparative.pathology@mcgill.ca

10.2. McGill Standard Operating Procedures (SOP)

<http://www.mcgill.ca/research/researchers/compliance/animal/sop>

10.3. University Animal Care Committee (UACC) online theory course

- In order to be approved on the animal use protocol, participant must complete the online theory course.
- Basic level: For participants performing techniques shown in Module 1 only.
- Advanced level: For participants performing techniques shown in Modules 2 and above.
- Link to theory course: <http://animalcare.mcgill.ca/>
- Email: animalcare@mcgill.ca

10.4. Photographing/filming guidelines

- McGill Social Media Guidelines: <https://www.mcgill.ca/newsroom/socialmedia/guidelines>
- UACC Guidelines for photography/filming of animals for research purposes: http://www.mcgill.ca/research/files/research/guidelines_for_photography_-_nov_2015.pdf

10.5. Useful links

- Canadian Council on Animal Care: www.ccac.ca
- Animals in Research and Teaching: <https://www.mcgill.ca/research/researchers/compliance/animal>

The UACC would like to acknowledge the invaluable help of the Comparative Medicine and Animal Resources Centre Animal Health Technicians in preparing this handout.