Welcome to the workshop

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I. Memarian

SHALTSPEARE

Goals

- Anatomy & physiology
- Housing and Routine Care
- Animal Identification
- Sex Determination
- Intravenous Injection
- Blood Collection
- Intraperitoneal Injection
- Oral Administration
- Subcutaneous Administration
- Anesthetics & Analgesics

introduction

Mice Name :Mus Musculus Order : Rodentia Family : Muridea *over 500 outbred stocks & inbred strains in the world Rat Name : Ratus norvegicus Order : Rodentia Family : Muridae

genetics

- > Outbred stocks => Heterogenous
- Inbred strains

Homogenous

after 20 generation, the probability of homozygosity at any gene locus is more than 98%.

F1 hybrids

Mice & Rat attribute

- Short life span
- Short gestation
- Large litter size
- Great genetic diversity
- Low purchase cost
- Ease of maintenance

Area of uses

HTN Neoplesia Teratology Toxicology Embryology Aging Etc

Behavior

Both of them are social

 Rat rarely fight & males can be housed together with few problems.

Anatomy & physiology

- Brown fat between the scapula & the ventral cervical region can be confused with salivary gland or lymph nodes
- They can not vomit
- Absence of gallblader
- Presence of diffuse pancreas, numerous salivary gland

Biologic & reproductive data for mice

Adult body weight

Male	20-40 g
Female	25-40g
Life span	1.5-3
Body tempratu	re 36.5-38
Heart rate	325-870
Respiratory rat	e 60-220
Food	12-18 g/100g/d
Water	15ml/100g/d

Breeding onset

Male	50d
Female	50-60d
Estrous cycle length	4-5d
Gestation period	19-21d
Postpartum	Fertile
Litter size	6-12
Weaning age	21-28
Breeding duration	7-9mo
Chromosome numbe	er 40

Biologic & reproductive data for rat

Adult body weight

Male	300-520 g
Female	250-300g
Life span	2.5-3.5
Body temprate	ure 35.9-37.5
Heart rate	250-450
Respiratory ra	ite 70-115
Food	5-6 g/100g/d
Water	10-12ml/100g/d

Breeding onset

Male	65-110d
Female	65-110d
Estrous cycle len	gth 4-5d
Gestation period	21-23d
Postpartum	Fertile
Litter size	6-12
Weaning age	21
Breeding duration	n 350-440d
Chromosome nur	mber 42

Housing and Routine Care: Flooring

Rodent caging has two types of flooring:

Solid

wire mesh



Housing for Mice





Routine Sanitation

What's the matter?

for protecting animal health

 for minimizing the introduction for nonexperimental variables which could undermine the quality of research data.

Environmental Controls

- room temperature
- humidity
- ventilation
- illumination and light schedule
- noise abatement

Animal Handling and Restraint



One-handed Method

Follow the above steps.

Then, place the mouse's tail between the last two fingers of the hand that is holding the mape.

HOW TO PICK UP A MOUSE

When you handle mice, all your movements should be slow and gentle. They are lively animals, but they will rarely bite unless they are afraid or mishandled.

Grasp the middle of the tail between your thumb and index finger, and lift the mouse.

NOTE: Frequently, the animal will hang quietly and can be carried in this manner. Occasionally, an individual will turn around and crawl onto the palm of your hand. It should be allowed to do this.

HOW TO PICK UP A MOUSE WITH THUMB FORCEPS



The thumb forceps are shaped somewhat like an eyebrow tweezer. The tips must be covered with rubber or polyethylene tubing to prevent damage to the mouse's tail.

Grasp the middle of the tail between the forceps and apply only enough pressure to lift the mouse.

CAUTION: Too much pressure may cause pain or even crush the tail.



DecapiCone[™] restrainer

Cylinder restrainer (flat bottom)





Reproductive Data Of Mice

Stage	

- Sexual maturity
- Estrous cycle
- Gestation
- Weaning age
- Reproductive life
- Life span
- Pups begin eating dry food

Age

40-60 days

- 4 5 days
- 21 days
- 21-30 days
- ±8 months
- 1 3 years
- 12 -14 days

Animal Identification

Ear punching or notching
Ear tags
Microchip transponders
Tattooing toes (neonates) or tails
Toe clipping

Laboratory Animal Handling Technique -Mouse

- A. Blood collection from tail vein
- B. Blood collection from orbital sinus
- C. Blood collection from cardiac puncture
- D. Blood collection from saphenous vein
- E. Intraperitoneal injection
- F.Subcutaneous injection
- G. Oral Feeding
- H. Sexing

Blood Collection From Tail in Mouse

 For collection of small amount of blood (Approximate 0.1 ml)

Tools for Blood Collection from Tail



- 75% alcohol cotton ball for surface disinfection
- Small plastic bottle with 1/2 cm diameter holes in both ends as mouse restrainer
- Scissors
- Pipetteman and tips
- A vial for blood collection



Placing a mouse on a cage lid and grasping the loose skin behind the ears by the thumb and forefinger



Push the mouse into the restrainer



Leave the tail of the mouse outside the cover of the restrainer



Amputate the tip of the mouse tail by scissors



Massage the tail and collect blood by pipetteman

Blood Collection From Orbital Sinus in Mouse

- Should apply anesthetic before blood withdraw
- A convenience and easy apply method for blood collection in mouse
- Collect amount up to 0.5 ml

Tools for Blood Collection from Orbital Sinus in Mouse



- 75% alcohol cotton ball for surface disinfection
- Hypnorm for general anesthetic
- 27 G needle with 1 ml syringe for injection
- Glass capillary tube and vial for blood collection



Anesthetize a mouse by intraperitoneal injection of Hypnorm



Use a sharp end glass capillary tube to penetrate the orbital conjunctiva and rupture the orbital sinus



Collect blood with a vial

Tools for Cardiac puncture in Mouse



- 75% alcohol cotton ball for surface disinfection
- Hypnorm used as anesthetic
- 27G needle with 1 ml syringe for injection
- 24G needle with 3 ml syringe for blood withdraw



Anesthetize a mouse by intraperitoneal injection of Hypnorm


Disinfect the thorax area with 75% alcohol cotton ball



Search for the maximum heart palpitation with your finger



Insert a 24G 1" needle through the thoracic wall at the point of maximum heart palpitation



Withdraw blood slowly by your right hand

Blood Collection From Saphenous Vein in Mouse

- This method is used of multiple samples are taken in the course of a day
- It can also be applied on rats, hamsters, gerbils and guinea-pigs

Blood Collection From Cardiac Puncture in Mouse

- For collect up to 1 ml of blood within a short period of time
- Must be performed under general anesthetic

Tools for blood collection from Saphenous vein in mice



- 75% alcohol cotton ball for surface disinfection
- 50 ml syringe tube with small holes at the end as restrainer
- a scalpel and shaver for remove of hair
- 24 G 1 " needle for release of blood
- tips and pipetteman for blood collection



Placing a mouse on a cage lid and grasping the loose skin behind the ears with your thumb and forefinger



Place the mouse in the restainer



Pull out the leg and removed the hair by a assistant



Hair can also be shaved by using a small scalpel



The saphenous vein is seen on the surface of the thigh



Apply vaseline after disinfect the surface area to reduce clotting and coagulation during blood collection.



Use a 24 G 1" needle to puncture the vein and release blood from the saphenous vein



Use a Microvette or a pipetteman with tip to collect blood from the saphenous vein



Approximate 100 microliters can be collected



Flex the foot of the mouse to reduce the flow of blood back to the puncture site



A cotton ball is applied to the puncture site to stop further bleeding

Intraperitoneal Injection in Mouse

A common method of administering drugs to rodents

Tools for Intraperitoneal Injection in Mouse



- 75% alcohol cotton ball for surface disinfection
- 25G 1/2" needle with 1 ml syringe for injection



Place a mouse on a cage lid and grasping the loose skin behind the ears with your thumb and forefinger



As soon as the mouse's head is restrained, the mouse can be picked up and the tail secured within your ring finger and little finger



The injection site should be in the lower left quadrant of the abdomen because vital organs are absent from this area. Only the tip of the needle should penetrate the abdominal wall to prevent injection into the intestine.

Subcutaneous Injection in Mouse

The most common method for immunology studies

Tools for Subcutaneous Injection in Mouse



- 75% alcohol cotton ball for surface disinfection
- 25G 1 " needle with 1 ml syringe for injection



Pick up a nude mouse and spin it's tail to put it in a faint condition



Grasp the loose skin on the back of the mouse from ears along the legs and restrain the legs with your ring finger and little finger



After disinfect the surface area, insert the needle in the lateral side of the abdominal wall and push upwards to the armpit of the mouse



Inject the substance slowly



A lump of injection substance can be seen through the skin after injection

Oral Feeding in Mouse

- Gastric intubation ensures that all the material was administered
- Feeding amount limited to 1% of body weight

Tools for Oral Feeding in Mouse



A 18 G stainless steel, ball tipped needle
a glove



Grasp the loose skin on the back of the mouse and restrain it's tail with your ring finger and little finger. Then, introduce the feeding tube from the pharynx in to the esophagus when the mouse is in the act of swallowing. Common complications associated with gastric intubation are damage to the esophagus and administration of substance into the trachea. Careful and gentle passage of the feeding needle will greatly reduce these possibilities.



Sexing mice - The distance between the anal and genital orifices is greater in the male (left) compared to the female (right).

Laboratory Animal Handling Technique -Rat

- A.Intraperitoneal injection
- B.Blood collection from tail vein
- C.Blood collection from cardiac puncture
- D. Oral feeding
- E. Sexing
Intraperitoneal Injection in Rat

 The most common method of administering drugs to rats

Tools for intraperitoneal injection in rat



- 75% alcohol cotton ball for surface disinfection
- medium size towel for restraining the rat
- 25G 5/8" needle with 1cc syringe for injection



Let the rat relaxes on the top of the lid.



Stretch the body of the rat by pulling up it's tail and then cover the rat with a towel by your left hand



Fold the skirts of towel under the rat from all directions



Grasp up the left hindlimb of the rat to expose the abdomen



The injection site should be in the lower left quadrant of the abdomen because vital organs are absent from this area



Only the tip of the needle should penetrate the abdominal wall to prevent injection into the intestines.

Collection of blood from tail vein in rat

General anesthesia needed
small amount: 0.1-1 ml

Tools for collection of blood from tail vein



- 75% alcohol cotton ball for surface disinfection
- 27G1/2" needle with 1 ml syringe for blood withdrawal
- a vial for blood collection



Optimal site of blood withdrawal is around the distal one-third of the tail since this part of tail gives better visualization of the veins



Disinfect the tail with 75% alcoholic cotton ball



When the needle penetrates the epithelium of the tail, pull back the plugger a bit to create negative pressure inside the syringe, then push the needle in the vein slowly until blood get into the dead space of the needle head



Pull back the plugger by the ring finger to withdraw blood from the tail vein



Using a scalpel to make a small wound on the tail is also an option for collecting blood from tail vein



Blood can be collected b using a pipetteman after then

Collection of Blood from Cardiac puncture in Rat

- General anesthesia needed
- Large amount: up to 3% of body weight

Tools for collection of blood from cardiac puncture



- 75% alcohol cotton ball for surface disinfection
- 24G needle with 10cc syringe for blood withdraw
- 15 cc centrifuge tube for blood collection



Disinfect the left thorax with 75% alcoholic cotton ball



Search for the point of maximum heart beat



Insert the needle straight on the selected point and withdraw blood by your left hand

Oral Feeding in Rat

 Feeding amount limited to 1% of body weight

Tools for oral feeding in rat



- 16 G ball-tipped feeding needle with syring
- Leather glove



Restrain the shoulders of the rat by your thumb and index finger, then support the lower limbs with your right hand



Restrain the tail of the rat in between your ring finger and little finger



Let the rat lying on your left palm and introduce the ball-tipped feeding needle from the pharynx in to the esophagus when the rat is in the act of swallowing



Sexing Rat- the distance between the anal and genital orifices is greater in the male (left) compared to the female (right)

sex Determination



Thank for your attention