April 29th, 2024

Anesthesia and analgesia in laboratory animals

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Care and Use of Laboratory Animals

10th International course

Pain

IASP definition:

- ” An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.”
- ”Pain is always subjective (…)”

IASP 1979

Nociception

The detection of a noxious event or a potentially harmful event

Vs PAIN = psychological and other responses to it

Why is it important to avoid pain?

For ethical reasons

• Since pain may be a source of suffering, we have a moral obligation to avoid it whenever possible

For scientific reasons

1. Stress response – altered physiology
2. Impaired recovery after surgery
3. Risk of development of chronic pain

Risk of animals in distress and suffering

Risk of bias to experimental data

1. Stress response

The body's response to stressors – factors that trigger stress

Major impact on physiological and endocrine functions

- Impact on experimental data
- Increased variation between subjects

With the risk of distress and suffering!

Acute vs **chronic** stress

Distress: an aversive state in which an animal is unable to adapt to stressors and shows maladaptive behaviours

Fear, anxiety and stress/distress increase pain

2. Impaired recovery after surgery

Superficial breathing – poor blood oxygenation

Cessation of intestinal function (nausea, vomiting, intestinal paralysis, endotoxaemia)

Difficult auto-regulation of body temperature

Dehydration and catabolism due to stress response, decreased food and water intake

slow healing

slower return to normal body functions

3. Development of chronic pain

Acute pain is necessary for survival

If it persists, central mechanisms may be altered at spinal or supraspinal levels – chronic pain (pathological)

Noxious stimuli produce an exaggerated and prolonged pain (allodynia)

Stimuli that would normally not produce pain, now begin to do so

This occurs during anaesthesia if proper analgesia is not provided pre- or intraoperatively

Pain and stress assessment

Physiological parameters

Heart rate

Blood pressure

Body temperature

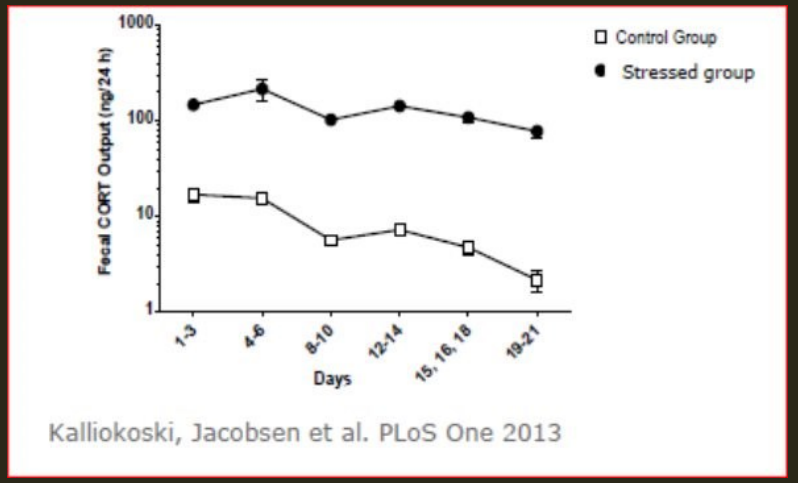
Biomarkers

Corticosteroids

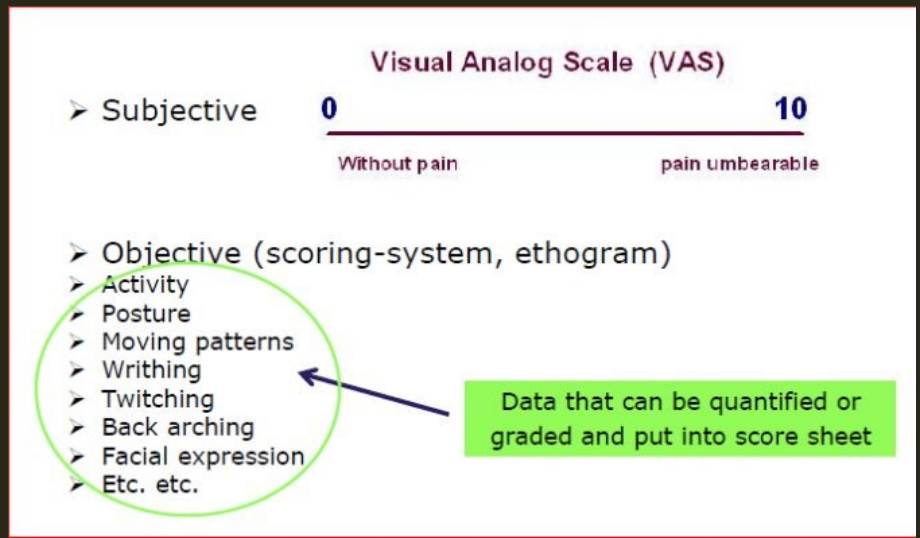
Adrenaline/noradrenaline

C-fos activation

Behavioural and clinical signs



Kalliokoski, Jacobsen et al. PLoS One 2013



Behaviour and clinical signs

How do we know if the animal experiences pain or distress?

Is the animal alert/moving normally?

Does it eat or drink more or less?

Does it bite or lick itself more than normal?

Is it protecting a certain part of the body?

Rats: chromodacryorrea (red coloured tears)

Acute pain: aggression, isolation, restlessness, self-mutilation, twitching, back-arching/dilated pupils, rapid breathing

Chronic pain: piloerection, isolation, hunched posture/weight loss, dehydration

Pica-behaviour

Take into account:

Basic normal behaviours (prey animals/peak activity during twilight and dark periods)

Nesting/hiding, social behavior, exploration, grooming

Behaviour and clinical signs

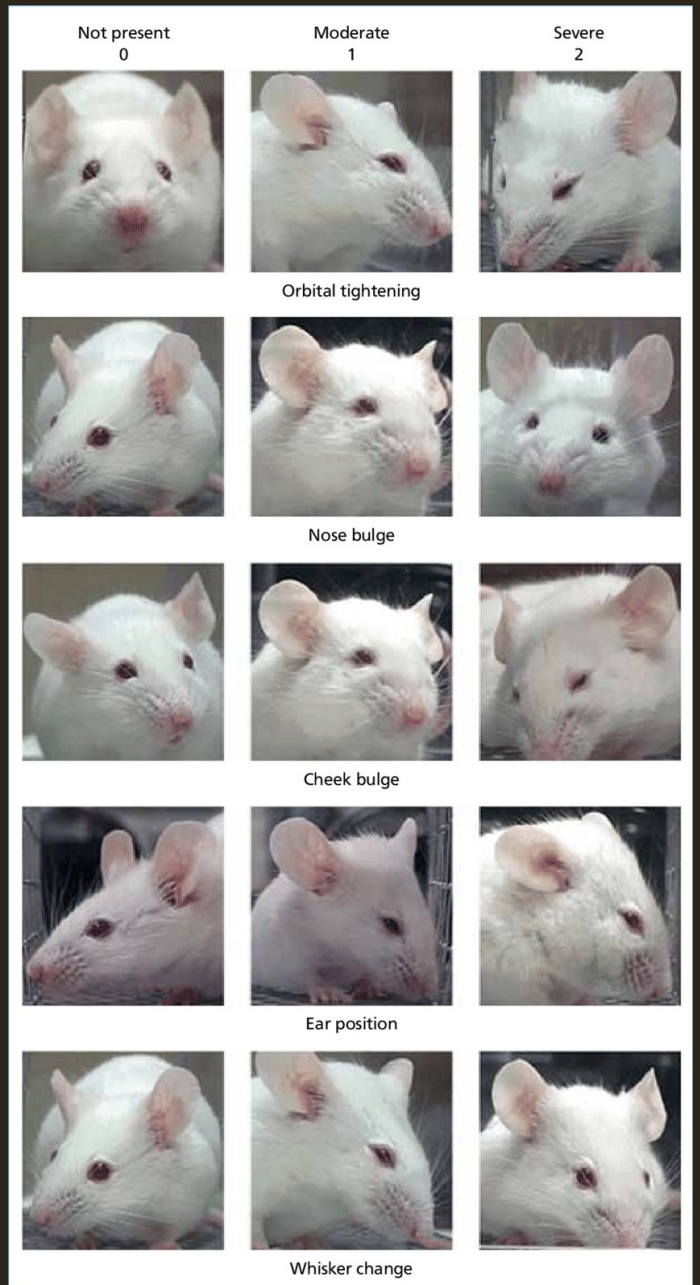
Body weight loss

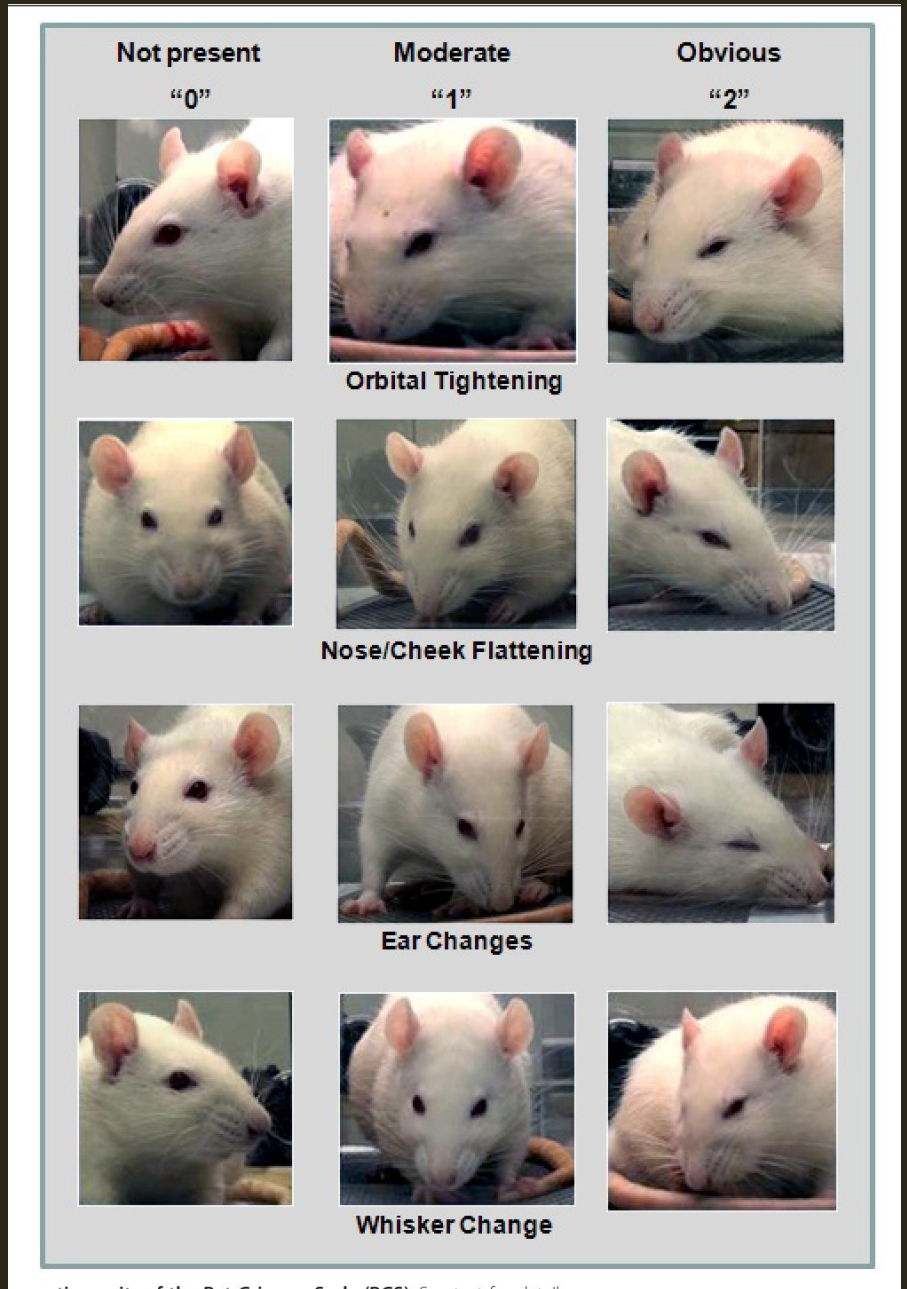
Food and water consumption

Urination/defecation

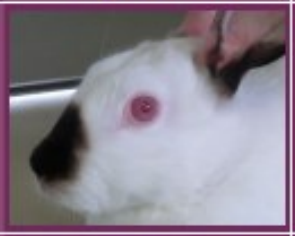
Fur quality

Facial expression





Facial expression

	Action units		
	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening - Closing of the eyelid (narrowing of orbital area) - A wrinkle may be visible around the eye			
Cheek flattening - Flattening of the cheeks. When 'obviously present', cheeks have a sunken look. - The face becomes more angular and less rounded			
Nostril shape - Nostrils (nares) are drawn vertically forming a 'V' rather than 'U' shape - Nose tip is moved down towards the chin			
Whisker shape and position - Whiskers are pushed away from the face to 'stand on end' - Whiskers stiffen and lose their natural, downward curve - Whiskers increasingly point in the same direction. When 'obviously present', whiskers move downwards			
Ear shape and position - Ears become more tightly folded / curled (more cylindrical) in shape - Ears rotate from facing towards the source of sound to facing towards the hindquarters - Ears may be held closer to the back or sides of the body			

Alleviation of pain

Anaesthesia

a state of controllable, reversible insensibility in which sensory perception and motor responses are both markedly depressed (partially or totally)

Microsurgery – micro pumps, micro dialysis, miniaturized imaging machinery, etc

Analgesia

the temporary abolition or diminution of pain perception

Aseptic conditions

- Always apply/use sterile conditions when performing surgery on rodents

- Although rodents are believed to be resistant to post-surgical infections, following stress or immune suppression, subclinical infections can develop to clinical disease and alter physiological data and behaviour.

Foster, *et al.,* 1982

Behavioral and Physiological Effects of Inapparent Wound Infection in Rats, Lab.Animal Science, 42(6), 572-578, 1992. Errata, Vol43 (2), 20, 1993

Aseptic conditions

Major rules

Sterile things must touch only sterile things!!!

Non-sterile things must touch only non-sterile things!!!

Tissue trauma and pain

Major rules

Surgery should be gentle – rough handling results in increased pain

Time is trauma – exposure of organs to RT/air is toxic. Stay under 90 min to keep possibility of infection minimal

Special considerations in rodents

Specific anatomic and physiologic peculiarities that influence the effects of anaesthetic drugs

- Small body size – high surface to body volume ratio
- Fast drug metabolism and excretion
- With high metabolic rate and limited fat storage, energy depletion can be stressful
- Doses of drug (per kg body weight) tend to be higher than in larger species
- Dehydrate faster per unit of time
- Reduced glycogen reserve – predisposes to hypoglycemia
- High oxygen consumption rate – mortality due to hypoxemia
- Lose body heat rapidly through hairless areas – frequent intraoperative mortality due to hypothermia

Peri-operative preparation

- Let animals acclimatize at least for 5-7 days prior to procedure
- Make sure the animal is healthy (normal posture, bright eyes, etc)
- Measure body weight before anaesthesia

Fasting

- Take away food, not water!
- Faster metabolism = shorter fasting
- Rats, guinea pigs and rabbits, up to 6 hours. No fasting for mice.
- Only guinea pigs can vomit – intubation may be required

Preanaesthetic considerations

- Small body size, high metabolic rate, hypothermia
- Core body temperature decreases during the course of general anaesthesia
- Strain, body weight, age and sex add to anaesthetic variability

Examples:

Mice <8w metabolize anaesthetics less efficiently than adults due to immature liver enzymatic system and reduced homeostatic response

Corticosteroids, sexual hormones, hepatic enzymes influence the pharmacokinetics and metabolism of anaesthetics

Obese mice present altered biodistribution of lipophilic agents

Premedication

Tranquillizers and analgesics to reduce apprehension, favour stress-free induction and recovery, reduce doses and side-effects of anaesthetics and achieve pre-emptive analgesia

Atropine: to reduce bronchial and salivary secretions and protect the heart

rodents 0.05mg/kg

Rabbits 1-3mg/kg (fast metabolism, use glycopyrrolate, 0.5mg/kg)

Approximately 30min before surgery

Anaesthetic regimen

A state of unconsciousness, analgesia, muscle relaxation and a-reflexia

Choice of anaesthetic? Minimal influence to study

Immobility without or with minimal pain

Administration? Inhaled or injected

Skilled handling

Pre-anaesthetic medication

- Anaesthetic at a dose of 20-50% of the final dose
- Easier handling if iv injection or intubation is needed
- If procedure not painful, but animal needs to be immobilized

Oxygen

- Respiratory tract is open
- Extra oxygen directly into mouth/intubation
- Respiratory rates:

Small rodents 50-100 breaths/min

Rabbits 30-60 breaths/min

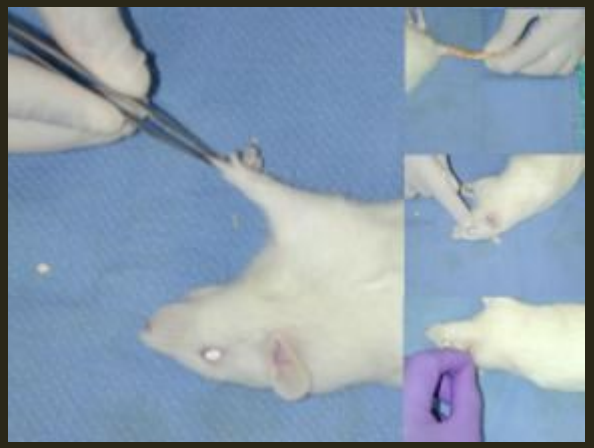
Larger species 10-30 breaths/min

- Reduction to less than 50% gives cause to concern!

Hypothermia

- Common cause of death
- Delays wake-up time
- Especially important in small rodents and birds
- Cold disinfection agent, saline, etc
- Warming lamps and blankets
- Careful to not overdo it!

Depth of anaesthesia



- Mouse

Respiratory rate

Cornea reflex

Tail pinch

Pedal reflex

- Guinea pig

Palpebral reflex

Ear pinch

Might move 2-3 times – does not reflect weakening of anaesthesia

- Rat

Respiratory rate

Tail pinch

Pedal reflex

Ear pinch

- Rabbit

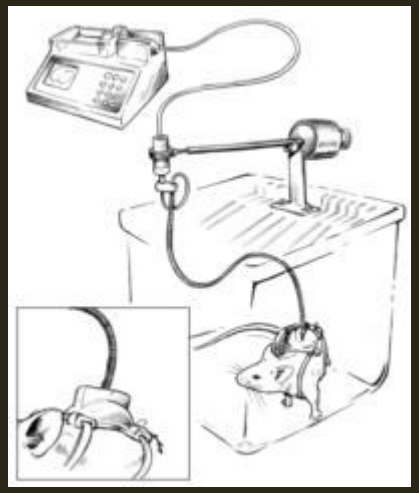
Light surgery – pedal reflex

Medium depth – palpebral reflex and ear pinch

Corneal reflex – dangerously deep

Conduction of anaesthesia

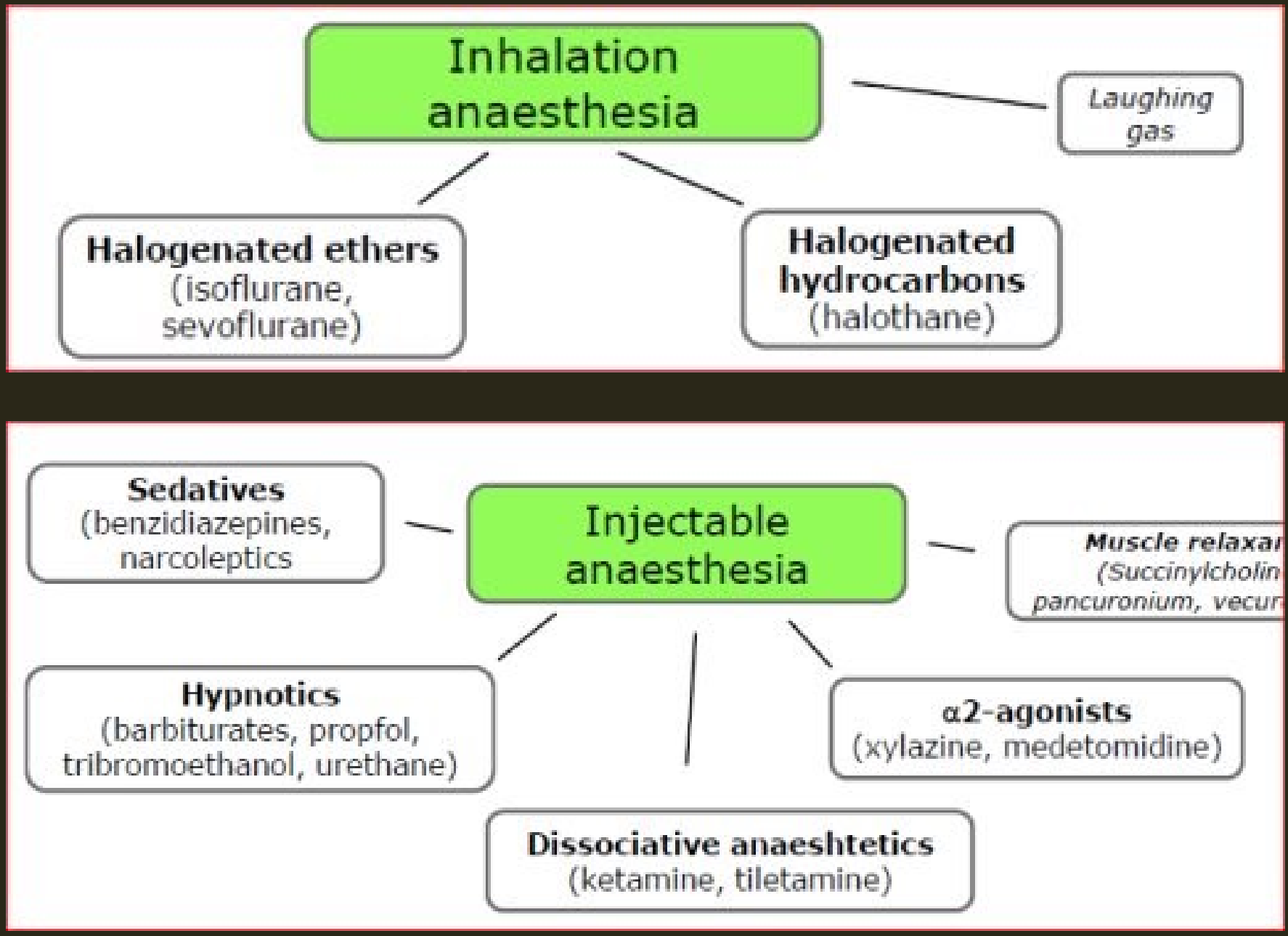
- Inhalation: easier
- Iv injection: dose is divided. The second half later
- Infusion: easily conducted
- IM, ip and sc: the entire dose is given in one administration, the reaction of the animal may vary



Inhaled vs injected

- Easier administration
- Safer
- Reduced impact on liver and kidney
- Rapid recovery
- Quick adjustments and easy maintenance of anaesthetic depth
- Respiratory and myocardial depression
- Vasodilation and hypotension
- Need for special equipment

- May require prior immobilization
- Controlled anaesthetic depth
- Controlled recovery
- No specialized equipment needed
- Cardiovascular depression
- Difficulties in dose calculation
- Reversibility by antagonists


Inhalation anaesthesia
Laughing gas
Halogenated ethers (isoflurane, sevoflurane)
Halogenated hydrocarbons (halothane)
Sedatives (benzidiazepines, narcoleptics
Injectable anaesthesia
Muscle relaxa
(Succinylcholin
pancuronium, vecur
Hypnotics (barbiturates, propfol, tribromoethanol, urethane)
α2-agonists (xylazine, medetomidine)
Dissociative anaeshtetics (ketamine, tiletamine)

Inhalation anaesthesia

Offers a wide margin of safety

Allows the maintenance of a constant plane of anaesthesia

Inhalation equipment: vaporizer, pulse oximeter and end-tidal CO_2, body temp control, blood gases, ECG

Small animals: induction chamber and maintenance of depth with face mask (size is important!) – Bain circuit (non-rebreathing)

Most commonly used inhaled anaesthetics:

halothane, isoflurane, sevoflurane and nitrous oxide

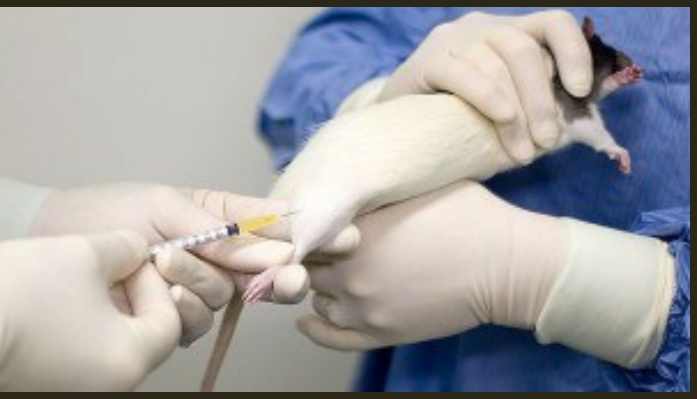
Injected anaesthesia

- Injectable anaesthetics can be best administered via IP, IM and IV routes
- The SC route is unpredictable for anaesthetic induction because of its variable and slow absorption rate
- Adequate volumes:

  IP: 0.1 – 1ml

  IV: 0.05 – 2ml

  IM: <0.05ml



Laboratory anaesthesia

- Avoid polytherapy – simple solution is the best
- Think of the influence to your study
- Prefer inhalation anaesthesia
- Prefer reliable anaesthesia

Post-operative care

- Room must be warm (27 – 30^{o}C) and quiet
- Fluid therapy:

  i.p or s.c 40 – 80ml/kg (e.g. mouse 1-2ml, rat 5ml)

- Extra oxygen
- Awakening chamber
- Reduction of heat loss

Ideal analgetic

- Effective pain-killer
- In line with “pre-emptive analgesia”
- Does not influence the study
- Does not cause depression
- Does not require frequent administration
- Weak effect on cardiovascular system and respiration

Analgetics do not substitute good surgical technique

do not reduce tissue damage!!

Advantages of analgesia

- Faster recovery
- Faster recovery of appetite
- No weight loss

Opioids, non-steroidal anti-inflammatory drugs or local anaesthetics may be used successfully in mice

Drugs	Dosage
Meloxicam (NSAID)*	1 mg/kg SC, PO 30 min presurgery and q24h postsurgery
Carprofen (NSAID)	5 mg/kg SC PO q24h
Ketoprofen (NSAID)	2-5 mg/kg SC q12-24h
Buprenorphine (opioid)	0.05-0.1 mg/kg SC q12h
Tramadole (opioid)	10-30 mg/kg IP or 1mL 5% solution in 150 mL of water
Lidocaine (local anesthetic drugs)	1-4 mg/kg or 0.4 mL/kg of a 1% solution

Antibiotics

- Never used as a replacement for good surgical and aseptic technique

Justified if:

- Surgery involves gastrointestinal or urogenital tract
- Surgery is performed on immunocompromised/immunodeficient animals
- Surgery involves infected area
- When risk of infection is high: procedure longer than 1 hour
  implantation of foreign body
  new surgeons

Antibiotics

- Use before surgery and continue for 2-3 days
- Rodents (hamsters, guinea pigs) and rabbits easily get enterotoxemia because of delicate intestinal flora
- Do not use the same type of antibiotics – leads to antibacterial resistance
- Contact the consulting veterinarian at the institution for advice!!!

Drug	Dose	Species
Ampicillin	Rat, mouse:50-150 mg/kg s.c. 2 x per day or 500 mg/l drinking water. Rabbit:25 mg/kg i.m. 1 x per day	Not for guinea pig/hamster
Dihydrostreptomycin + benzylpenicillin Streptocillin vet.	Rat, mouse:1 ml/kg s.c. 1 x per day Rabbit:0,1 ml/kg i.m. 1 x per day	Not for guinea pig/hamster
Oxytetracyclin fx terramycin vet.	Rat, mouse:100 mg/kg s.c. 1 x per day or 500 mg/l drinking water	Not for guinea pig/hamster
Sulfadioxin/ Trimetoprim fx Tribrissin vet.	Small rodents:1 ml/kg (24%)s.c. 1x per day Rabbit:0,1 ml/kg (24%) s.c. 1 x per day	All animals

References

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Thank you