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A REVIEW ARTICLE ON ANAESTHESIA AND ANALGESIA IN LABARATORY ANIMALS

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Abstract: Anaesthesia and analgesia are major components of the ethical practice in the use of lab animals in biomedical research. From these sources it is seen that these interventions are necessary under circumstances where it becomes essential to reduce suffering, discomfort and anxiety to the subjects being experimented on. Proper anaesthetic regimens promote humane handling of animals that are subjected to surgery or other procedures requiring invasion of the body. There are many different anaesthetic agents with inhalational, intravenous and other types of anaesthetics with the choice dependent on species, size, age, overall health and specific needs of the procedure. Analgesia is equally as important as anaesthetics being the relief of pain before, during and after surgery interference. Pain relievers include NSAIDs, opioids and local anesthetics, and they help the animals to free from severe pain that threaten its welfare. New modalities have also added to multimodal analgesia, a technique that aims at using several types of analgesics to have better pain relief than a single one without side effects. Considering depth of anaesthesia and quality of analgesia is especially imperative in terms of animal welfare and possibly for contribution to experiment results. Both physiological and behaviour monitoring methods are used to identify the correct plane of anaesthesia and analgesia. However, legal requirements and ethical concerns define protocols related to the use of anesthesia and analgesia in laboratories. Specifically, animal reviewers belong to an Institutional Animal Care and Use Committees (IACUCs) of an institution and their function is to provide a check that a proposed or ongoing research use animals in a humane way, minimizing the levels of pain and suffering as much as is feasible.

Keywords: Anaesthesia, analgesia, laboratory animals, pain management, humane treatment, multimodal analgesia, ethical research, animal welfare.

1. INTRODUCTION

Anesthesia is widely used in animals as their inability to co-operate with certain procedures such as treatment or disease diagnosis. It can be simply defined as loss of sensation in animal to painful stimuli with or without loss of consciousness. The main purpose of providing anesthesia is to immobilize the animal and prevent the perception of painful stimuli while maintaining physiological functions of the animal as normal as normal as possible.

Good unaesthetic drugs facilitate smooth induction and recovery of animals that inhibit secretions, bronchial and salivary pre-operative pain in animals. Agent that gives short duration of anesthesia indicates half to one hour anaesthetic effect Anesthetic with durations of sleep anesthetic duration. [1]

Sedation, relaxation of skeletal muscles and pain relief are three fundamental bases in anaesthesia.The animals used in laboratories need to be anesthetized before undergoing through painful procedures such as cardiac deliver anesthetic agent either through inhalation or injections. [2]

Objectives of Anaesthesia

- For a purpose of operating on someone an all-important research function
- Ask for a physiological stability for the procedures
- Help the clients get back to par especially after the intervention process is complete.
- To minimize the cause of pain and discomfort during the process
- To offer immobility (to leave the catheter etc.)
- As precondition for the surgery (Muscle relaxation)

1.1 Pre-Anaesthetic Consideration and Pre-Anaesthesia

1.1.1 Pre- Anaesthetic consideration

1. Animal species and strain

Strain and species differences of laboratory animals in their reactions to anesthetics. Metabolic rates, physiologic

reactions and tolerance level differ among the individual necessitating appropriate anesthetic regimen. [3]

2. Health status and physical condition

The health of the animal should be assessed before surgery to determine if it has any conditions that may worsen tolerance to anesthesia (e.g., respiratory and cardiovascular diseases). [4]

3. Fasting

When a local anaesthesia may be required, fast for some reason may not be as severe in some species as in others. For instance while mice and rabbits are at high risk of hypoglycemia they do not have to be starved while dogs or pigs on the other hand have to be starved in order not to aspirate. [5]

4. Age and Weight

As we shall see, age and weight are critical factors when it comes to using anesthesia. If the animal is a juvenile or neonate for example a young calf, pup or lamb the client should inform the veterinarian so that right dosage can be adopted to prevent toxicity. [6]

1.1.2 Pre -anaesthesia

Pre anaesthesia or pre medication is beneficial to both the anesthetist and the animal and makes the anesthetic induction and maintenance easier on part of the anesthetist and safer and comfortable on the part of the animal. Pre-medication is normally administered before interventional procedures for instance surgery work well in lessening the patient fear and in anticipation of general anesthesia.

Sedation: Sedation has a quieting influence on animals which must reduce their motility and render them unconscious of their environment. Though it causes hypothermia when used as a premedicant before inducing anesthesia and also decrease the amount of injectable used for anesthesia by 20-50%.The main reason for premedication is to minimize on anxiety and to prepare a patient for general anesthesia.

Classification of pre - anaesthetic drugs

a) Anticholinergic

Anticholinergics were given preoperatively to reduce salivary and bronchial secretions.sed in premedication to decrease the salivary and bronchial secretions. These drugs can block parasympathetic stimuli e.g.: Atropin sulphate, glycopyrolate. At times there is a need for minimizing secretions in bronchi to avoid blockage of airway during sedation in animal.

b) Sedative Anaesthesia

It causes a deeper state of analgesia having more dormiveness and inhibits both unconditioned instinctual and conditional reflexes.Phenobarbitone,Barbitone,Chloral hydrate, Benzodiazepines–Diazepam, Alpha 2 agonists– Xylazine, Detomidine. [7]

c) Sedative tranquillizer

Sedative tranquillizers cause a calming effect and are nonsedating, but they're not analgesic, so the animal will react to pain stimuli. It turns that sleep is induced by sedatives, which eliminate fear that is effective only for conditioned responses. Arousal reaction on sensory stimuli for instance visual, auditory, tactile or painful stimuli may be normal or hyperophic.e.g Phenothiazines: Promethazine, promazine, acepromezine, chlopromazine

Thioxanthines: Chliprothixene. [8]

1.2 ANAESTHESIA

They are classified into:

a. General anesthesia

b. Local anaesthesia

a. General anaesthesia

• Drugs produce reversible loss of sensation & consciousness, they administered by different routes: for the most part, inhalation and intravenous use.

• The drugs which are given as anesthetics by I.V administration are used for specific purpose as quickly acting general anesthetics for few minutes or as a supplement with another general anesthetic agents as: I.V anesthetic agent (thiopental), or Inhalation agents (nitrous oxide, ether & halothane).

Classification of general Anaesthetics

1.Inhalation Anaesthetics:

A. Volatile (liquid at room temp): are Halothane, Desflurane, Enflurane, Isoflurane, Methoxyflurane, Sevoflurane and Ether while the current available Inhalation Agents are Chloroform.

B. Gas (with a boiling point below room temp): are Nitrous oxide, Cyclopropane, Ethylene.

2. Intravenous Anaesthetics: Thiopental / barbital, Midazolam / benzodiazepines, Etomidate, Propofol, Ketamine, Fentanyl / opioid. [9]

Mode of action of general Anaesthetics

• Inhalation general anaesthetics→ banned nicotinic receptors and reveals K+ channels that hyperpolarize the neuron decreasing neuronal postsynaptic excitability.

• Intravenous general anaesthetics → act synergistically and enhance the presynaptic inhibition of the GABA neurotransmitter on GABAA receptor and opening of chloride ion channel challenging the postsynaptic neuronal excitability.

• Ketamine → inhibits NMDA receptors & counterpointed the excitotoxic action of glutamate neurotransmitter thus reducing post synaptic neuronal firing rate.

• Increase functionality of the inhibitory glycine receptors in the spinal motor neurons. [10]

b) Local Anaestheics

There are drugs that cause reversible loss of sensation on a given part of the body by blocking nerve transmission. Occasionally referred to as local anaesthetics, as they cause loss of sensation of pain without interfering with the nervous control. An ideal local anesthetic is one that is soluble in water, has no contact with nerve substance coulee irritant; must possess speedy action reaching its prime by the time the operation starts; should not be injurious when administered loco or generally; its action should be of a convenient duration for the operation.

Classification:

A. Injectable LAs:

1. Low potency, short duration: Procaine Chloroprocaine
2. Intermediate potency and duration: Lignocaine, Prilocaine
3. High potency, long duration: Tetracaine, Bupivacaine, Ropivacaine, dubucaine.

B. Surface LAs

1. Soluble: Cocaine, Lidocaine, Tetracaine
2. Insoluble: Benzocaine, Butyl-amino-benzoate, Oxethazaine. [11]

Mechanism of Action:

The LAs interact with the LA receptor present in Voltage gated Na+ Channel and either stabilise the channel or prolong the conduction in inactivated state and raise the threshold of channel opening and subsequently results in impulse conduction. [12]

1.3 ANALGESIA

An analgesic or painkiller is any number of group of drugs that are used to develop analgesia i.e. the absence of pain.

CLASSIFICATION

1. Opioid analgesics

a. Natural: morphine, codeine

b. Synthetic: Fentanyl, buprenorphine

2.Non opioid Analgesic

a. NSAIDS: Small dose of aspirin, or ibuprofen, diclofenac.

b. others: paracetamol

Mechanism of action of opioid analgesic:

As mentioned, drugs bind to opioid receptor reduces the activity of adenyl cyclase, which increases potassium conductance by opening potassium channels and decreasing calcium channel opening that decreases the neuron's excitability. Can cause hyperpolarisation and shows inhibitory pathway and release pain.

Mechanism of action of non- opioid analgesia:

The drug results in pain relief, and suppression of inflammation and fever due to blocking of prostaglandins synthesis. [13]

Aim: The underlying goal of anaesthesia and analgesia in laboratory animals is the human handling of those animals with least stressing them while at the same time maintaining balance in their physiological processes as affected by the experiment. This serves to promote ethical research and humane care of animals which are also used in research.

Objectives: The goals of anaesthesia and analgesia in laboratory animals include control of pain, stress, and safety of procedures, and physiological homeostasis, as well as properly coordinated recovery and complete adherence to the ethical norms concerning animal treatment.

2. LITERATURE SURVERY:

2.1. Cicero, L., Fazzotta, S., Palumbo, V. D., Cassata, G., Ignazio, A., & Monte, L. (2018). Anesthesia protocols in laboratory animals used for scientific purposes. 89(3), 337–342:

A suitable, effective and free of complications anesthetic protocol is very important in experimental studies on animal models since it could bias the outcome of a trial. To the date there is no universally accepted protocol for induction, maintenance and recovery from anesthesia.

2.2. Hawkins, P. (2014). "Recognizing and Assessing Pain, Suffering, and Distress in Laboratory Animals:

A survey was carried out to evaluate how animal pain, suffering and distress. Clinical signs used as indicators of potential pain, suffering or distress are largely subjective. The survey also addressed protocols and methods for avoiding and alleviating adverse effects, record keeping, review of policies and protocols and issues relating to team work and training.

2.3 Flecknell, P. A. (2015). Laboratory Animal Anaesthesia (4th ed.). Academic Press:

The last decade has seen continued progress in both the recognition and management of animal pain. This upsurge in the use of analgesics in animals is welcome, but the main areas of use continue to be the control of postoperative or post-trauma pain, and the management of musculoskeletal pain, in companion animals. Apart from providing some interesting parallels with pain management in people, development of veterinary pain management has potentially much greater significance. For many years, animal pain management has benefited from the use of analgesics used in man.

3. ETHICS AND SCIENTIFIC CONSIDERATION

3.1Ethical consideration:

3.1.1. Humane Treatment and Welfare:

It is common to involve a laboratory animal in studies to promote the area of medicine. Nevertheless, the treatment of these animals as ethically is very important. Most principles like the 3R's- Replacement, Reduction, and Refinement all come with the aim of lessening the pain experienced by animals. Effective anesthesia and analgesia are relevant to this goal which will eliminate the suffering of the animals during experimental procedures.

3.1. 2. Balancing Scientific Need with Animal Welfare:

Even if animals are required in some research for the explanation of some biological processes it is necessary to measure the advantages of such usage with the need to protect animals. For this reason, researchers have an ethical duty to demonstrate why they have used animals and why they should be treated properly.

3.1.3. Regulatory Oversight:

International bodies and institutions like the IACUCs ensure that animal used in research underwent through strict supervision of research protocols. The operating theatre committees also assess the use, or otherwise, of anesthesia and analgesia for various procedures in consideration of ethical considerations.[14]

3.1.4. Bioethics and the Concept of Sentience:

In cognitive ethology and neuroscience, there are emerging new studies patting forward the conviction that many animals are conscious and willing to feels various emotions like fear, pain and stress. The understanding of animal awareness is becoming more widespread and strong as a moral requirement to use anaesthesia and painkillers during an experiment.

Current controversies rise to the question of whether some kinds of studies can be undertaken whatsoever if the sentient beings must suffer painful procedures.

3.2 Scientific considerations

3.2.1 Refinement of Anaesthesia protocols

• The development of new technologies in veterinary anaesthesiology such as use of target-controlled infusion (TCI) have been depicted as a method that enables the delivery of anaesthetic drugs in animals according to their respective physiological characteristics. Anaesthetic agents in animals, based on their specific physiological parameters. TCI minimizes the incidence of wrong dosage of anesthetic agents thereby promoting animal welfare being in tandem with experimental behaviors.

• Local Anaesthesia: The practice of using local anaesthesia whilst incorporated with general anaesthesia has also interested researchers as a process of lowering down the general anaesthetics dosage and therefore reducing systemic side effects. This is particularly useful in the procedures where general anaesthesia alone may precipitate significant respiratory or cardiovascular compromise such as in rat models.

3.2.2. Drug-Specific Considerations:

• The choice of anaesthetic agent is crucial in maintaining the scientific integrity of experiments. Several anaesthetics, for instance, ketamine, cause cardiovascular and respiratory depression, which may confound outcomes in investigational research targeting such systems. As such, scientists may require performing preliminary experiments to establish which anaesthetics and analgesics cause the least interferences to the particular biosystems of interest.

• Species Variability: This is so because species differences in drug metabolism are a noteworthy factor. For example xylazine and ketamine is preferred for small mammals such as rodents but when used in rabbits and guinea pigs it results in extreme respiratory

3.2.3. Impact of Pain on Experimental Outcomes:

The biological stress response induced by pain has far-reaching effects, including changes in gene expression, immune function, and behaviour. The researchers have indicated that in some scenarios, chronic pain or stress can actually change the outcome significantly across the entire study in instances that involved immune system response, inflammation issues or neurological testing. Although anaesthesia may be employed during the procedure, inadequate post-surgery analgesia may introduce both stress-related responses, which will distort the outcome. For example, study discovered that with uncontrolled post-operative pain brings about hyperalgesia and allodynia which changes perceiving of results in pain study as well as other areas of study. Real times supervising of animals have been enhanced especially when in anesthesia and hence an improvement in the well-being of animals as well as enhanced accuracy in the results obtained.

3.2.4. Advances in Monitoring Technologies:

The refinement of real-time monitoring technologies for animals under anaesthesia has led to better outcomes in both animal welfare and data accuracy. Supravital observations of cardiopulmonary rate and rhythm, blood pressure, blood gases, and pH enable early detection of patients' intolerance to anaesthetic doses, and appropriate modifications are instituted. Progress in other types of measurement (e.g., infrared spectrum analysis and telemetry) helps to limit the use of additional invasive techniques and increases the proportion of premature animals' survival, especially when carrying out the long-term experiment. Equal importance must not be paid to the control of postoperative pain as the intraoperative management.

3.2.5. Post-Surgical Recovery:

Providing proper analgesia during the recovery phase is just as crucial as intraoperative care. Patients' pain at the site of surgery if inadequately managed leads to post-surgical complications, which include infections, slower wound healing time and development of behavioural changes thus may contribute to variability in the experiment.

Multimodal Analgesia: Clinicians are gradually beginning to practice multimodal analgesia; the use of two or more agents with varying mechanisms to treat pain in order to minimise on side effects and maximise on the effectiveness of the drugs. For instance, co-administration of NSAIDs with opioids or those local anaesthetics with systemic agents will decrease the unit dose of each agent and hence lowering. [15]

4. GENERAL PRINCIPLES

4. 1. Pre-anaesthetic Evaluation

Species-specific considerations: It is possible there are species differences in the physiologic reactions to anaesthetic drugs.

Health assessment: Take into account conditions that may arise due to age, weight or with the presence of any sometimes conditions when animals are to be anaesthetized.

Fasting: For example some species are deprived of food before anaesthesia for example some mammals whereas some species such as rodents does not apply since they have fast metabolism.

4.2. Choice of Induction and Maintenance Agents

Balanced anaesthesia: Employ synergistic drug therapy to provide the effect of unconsciousness, muscle relaxation and adequate analgesia. This minimizes adverse effects of each agent.

Species-specific drugs: It is important to understand that not all the anaesthetics are good for every species. Therefore, while ketamine is often applied with the rodents, it is possibly not suitable with larger animals.

Route of administration: Select depending on species, type of procedure & equipment available; this may include intravenous, intraperitoneal, inhalational etc.

4.3. Analgesia

Pre-emptive analgesia: Preoperatively, the use of analgesics should be employed in order to ensure that pain pathways are not set up in the first place.

Multimodal analgesia: Use opioids, NSAIDs or local anaesthetics to manage different types of pains whose causes are managed differently by the different drugs.

Duration of action: Minimise the time of inability to feel pain among clients by making sure that analgesia takes a cover as long as the operation is ongoing and up to the time of the operation's after-effect.

4.4. Monitoring during Anaesthesia

Physiological monitoring: Check pulse rate, respiratory rate, temperature, and SpO2 level at least every five minutes. What still needs to be unravelled are species-specific reference values.

Depth of anaesthesia: Ocular or limb withdrawal reflexes should be tested to assess the correct depth of anaesthesia and the

levels adjusted accordingly.
Thermoregulation: Small cracks in the skin, fur and body surface makes many animals especially small rodents to lose body heat when under anaesthesia, therefore, they need to be warmed.
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4.5. Post-Anaesthetic Care

Recovery monitoring: Check animals during their recovery for any signs of pain, low body temperatures or any other form of strange behaviour.
Postoperative analgesia: These can be continued with more rigorous protocols for intermittent intravenous analgesics during treatments requiring painful manipulation.
Hydration and nutrition: Make sure that animals can take their feed nicely once they are through with the rehydration process.

4.6. Reducing Stress and Distress

Acclimatization: Give animals some time before anaesthetic procedures so that they can adapt to the environment.
Handling: Appropriate handling procedures especially in the case of small animals such as rats and rabbits will help in minimizing stress and enhancing the results.

4.7. Ethical and Regulatory Considerations

Regulatory compliance: Follow IACUC policies and procedures and rules, and laws Humane endpoints: There should be specific limits put in order to determine at what stage a procedure should be ceased causing further pain or suffering.[16]

5. ANESTHETIC EQUIPMENTS

5.1. Anaesthetic Chambers:

When anaesthetizing small animals, it often proves simplest to use an anaesthetic chamber. Newer agents that can be administered employing a specific vaporizer are volatile anaesthetics and waste anaesthetic is evacuated by a gas-scavenging system. Because all anaesthetics have an effect of depressing the respiratory rate, the carrier gas should be oxygen instead of air, or oxygen and nitrous oxide. A clear Perspex box of suitable dimension should be used in this procedure, for instance, so that the animal has to be observed during induction. As is the case with sheep and goat handling systems, chambers can be bought from manufacturers or built from scratch, known as 'built-in'. Some chambers-being equipped with a metal grid on the bottom so that the animal is standing over the urine it produces. Otherwise, one of towelling or dry bed or some paper towels should be placed at the floor of the chamber. Washing after use is necessary and the apparatus should be cleaned comprehensively. The waste anaesthetic gas should not be expelled into the room in an uncontrolled manner and should either be ducted out of the room or adsorbed by activated charcoal. The common scavenging techniques among birds have been adapted in a special technique that involves two boxes

5.2. Face mask:

It will be shown that cone shaped face masks manufactured for veterinary use will be suitable for sheep pigs dogs cats and rabbits as long as the correct size is employed. This kind of mask should encircle the muzzle part appropriately, and it must not cover either the mouth or the nose part. It is also important not to use a mask that is too large as the space between the mask and the animals nose and mouth (equipment dead space) can retain exhaled gas, high in carbon dioxide and this will be inhaled unless extremely high gas flows are used to remove it. However, because even the lowest measurable flow rates that many anaesthetic machines can deliver are higher than actual rates needed by small rodents, most of the systems utilizing face mask work as open systems and dead space in the mask is rather insignificant. Small, light colorless masks with an accommodated flexible rubber diaphragm are convenient for some varieties of animals and birds. A mask design, removal of waste anaesthetic gas to protect the operator has been mentioned and this mask is available in the market. There are also several other system where gas scavenging is compounded with the delivery of the anaesthetics. On the regular use of face masks, it is recommended to wash the masks in warm water with soap as well as rinsing them before drying. Few of the mentioned can be autoclaved, but some of them may be sterilized by ethylene oxide.

5.3. Endotracheal Tubes:

Endotracheal tubes are applied to keep the airways patted open in order that breathing may be helped if required. They also safeguard the airway when the swallowing and coughing reflexes are held in check or are absent, so that a substance such as saliva does not go down the trachea. Endotracheal tubes are made by many manufacturing companies and come as plain tubes or those are supplied with an occlusive cuff that fits tightly against the wall of the tube and the trachea. The cuff can be inflated by a syringe (2 – 5 ml) or the OMRON Cuff Inflator for use with digital blood pressure monitors only. In the case of using MP, the cuff is blocked from deflation by the use of non return valve or by clamping by haemostats. Tubes maybe used several times or maybe planned for use only once. Original tubes are normally recyclable and are normally made from rubber; these tubes cannot be transparent. They are worst as they are not so flexible, are easily prone to breakage and also, are kink prone. The cuff tends to get compromised and lead to leakage; thus, it is possible to buy disposable tubes and let limited tubes be used repeatedly. Quite transparent polyethylene tubes have certain superiorityities out of which, condensation appeared in the tube with each breath gives an immediate feedback that is the tube is correctly placed in a breathing passage. Many of the tubes currently on the market are far too long for use in animals, the length of the tubes should be adjusted to cut down on dead space. When the animals are intubated and the head and neck flexed the likelihood of the tube becoming kinked increases. This can be prevented by using an armoured tube which is reinforced with a wire coil. Tubes should be check thoroughly before using them, in order to avoid adding a weak tube with a patient. Cuff must be also inflated to ensure there is no tear on the cuff and also to check that the cuff inflates properly. It should be washed in hot soapy water after use, and then rinsed well under clean running water and well dried. However, if equipment for pasteurization is capable then tubes can undergo the pasteurization process. However, many types do not withstand autoclaving, though some may be autoclaved a few time at low temperatures (121 degree Celsius for fifteen minutes), or sterilized using ethylene oxide. [17]

6. ROUTES OF ADMINISTRATION

6.1. Intravenous:

Intravenous injection of anaesthetic agents is also relatively easy in large animals, however within the small rodents this is complicated. An IV injection has got no absorption phase and hence has got immediate effect An IV can be dosed to effect according to an individual response unlike the situation in IM or SC or IP injection. Intravenous intermittent bolus dosage of

short-acting compounds like propofol, remifentanil allows dosing and dose rate control of the depth of anaesthesia. Another advantage of I reproducibility is if any emergency drugs are required they can be easily administered through i.v. In IV injection/infusion also supports rapid buffering of anaesthetic solutions that are poor, strategically either acidic (ketamine) or alkaline (barbiturates) that would cause tissue damage and pain if subjected to another route of formation. A study showed that propofol stimulates local pain at the site of injection although this pain can be effectively controlled by pre medication with an opioid or an alpha-2 agonist.

6.2. Intramuscular:

This route of administration could be administered in rabbits and larger species, but not in rodents since it may cause tissue injury. That is, an IM injection of K/X may lead to tissue necrosis and pain in the larger species in the same manner been described and analyzed above.

6.3. Intraperitoneal:

The reason why this injection route is chosen may be that intravenous access may be difficult and they simply do not have big muscles. Peritoneum is an extensively well-perfused organ and the drug kinetics after injecting small volumes of the drug is quick. One of the administered solution will flow through hepatic portal blood to the liver before entering the systemic circulation thus undergo high first pass hepatic metabolism. One of the dangers of IP injections is that some part of the injected solution may go to the stomach or intra abdominal fat and so may not work. [18]

6.4. Subcutaneous

The use of anaesthetics through the SC route can also be used instead of IM or IP injection. The rate of small volume absorption often does not show significant differences between the routes and SC injection seems to be less stressful or painful compared to IM injection in rabbits. Examples of anaesthetic agents given SC include ketamine/medetomidine in rabbits and rodents and midazolam in rats. Compared to the latter combination, the SC dose of the latter is one-third the IP dose. [19]

7. LABARATORY ANIMALS

7.1 RAT

The principal species employed in research are brown or Norwegian rat, a member of the Rattus norvegicus family. There are outbred and inbred strains available. Some of the popular outbred stocks are Wistar and Sprague –Dawley rat strains. There are fewer inbred strains than for the mouse, but one example which the reader might come across is the Lewis rat.

Figure 1: Norwegian rat

7.1.1 Behaviour

Rats are gentle and cooperative animals especially when treated well, however,, there are differences rooted in breeding strains. They will grow more friendly with regular interaction. They are social animals and will cohabit in a large population with almost no conflicts provided the population density is not pegged. It is characteristic of rats to be nocturnal rodents. Eating, drinking and copulation are activities mainly exercised at night. They are also weak-eyed and when the eyes of rats become blind they can go about as if nothing is wrong.

7.1.2 Housing

Rats can be housed in metal and plastic cages there is controversy about using mesh floors without bedding. When mesh floors are applied it is very important that mesh size is such that young animals cannot fall through it but, at the same time, the rats cannot catch their feet on the mesh. Mesh floors are not recommended in breeding females as nesting cannot be done. In solid bottom cages paper based commercial bedding, wood shavings or corn cobs may be used as bedding. According to type of used bedding, the cage should be changed at least weekly; cleaning routine will depend on the stocking rate. Standing seems to provide rats entertainment and therefore the cages must be high lid.

7.1.3 Feeding

It has to be mentioned that all the rats like other rodents are involved in the coprophagy. They can be fed ad lib on a complete pelleted rodent diet and should be received from hoppers placed over the floor of the cage. The use of food hopper should be washing at least once or twice in week. It is appropriate that the diet should contain between 20 and 27 percent protein. Protein levels beyond this may decrease reproductive perfromance. Rats are very selective with the food they take mostly avoiding new food items. Rats will consume feed from 5-10 grams for every 100gm of body weight in one day.

7.1.4 Water

Drinking water may be available by sipper tubes or through the use of watering equipment. The system need to be cleaned at least once, and preferably twice a week. The water may be needed to be made acidic or contain chlorine to minimize the level of contamination particularly for immune compromised rats. Rats will consume on averagely 10ml water per 100 g body weight daily.

7.1.5 Environment

Rats are much less thermoregulatory as mice but they are recommended to be kept in the temperature ranging from 19°C

to 23°C. Newborn rats are endowed with plenty of brown fat for performing thermogenesis and the amount decreases as the rats grow older. The humidity should be 40% – 70%. Low humidity causes tail ring, where an annular lesion is formed around the tail and can lead to sloughing of the tail beyond the lesion. For rats the light period needed is 12 hours but as the rat is nocturnal, light is harmful to them, and even more-so for albino rats which ultimately causes retinal degeneration. The level should be less than 400 lux but 100 lux for the albinos. Photoperiod influences the oestrus cycle and the best condition for successful breeding is found to be 12–16 h lighting period. Rat pathogen related aerosols require ventilation because several of the pathogens carried by rats are transmitted through the air. The rate of ten to twelve and fifteen changes an hour is acceptable but if the air is recycled or no filter is installed then it is not allowed.

7.1.6 Growth

Growth in male rats is longer, and bones do not mineralize fully until the animal's 2nd year. The growth characteristic of a rat varies slightly between the inbred and the outbred rats.

7.1.7 Handling

This is good because there are many ways of handling rats. Rats are tractable animals and they seldom bite if handled properly though there are differences in this regard depending on strain.

1. Take off the top of the cage and take the rat firmly by the base of the tail. The animal should be lifted from the cage and placed on a non-slippery surface, fanning the animal on the arm or cage top. Rats undergo severe strain, tension and stress should they be caught by the tail, thus should not be hung for any length of time. If the animal was born previously on a surface, the animal can be sexed by displacing the tail to show perineum, as of rat.
2. To restrain an animal for examination or other procedures, having placed it on a non-slip surface as above, slip the hand up its body and position it behind the shoulders of the animal. This should be done by placing the thumb, behind the other forelimb placing it under the chin to keep the head still while the fingers are placed at the back of the other forelimb. The animal may then be lifted and the hind quarters supported in a like manner with the other hand
3. If more control is required for added restraint complete step 1 then using the right hand put the other hand up the rest of the body and press the elbow with the thumb and fingers to cause the forelegs to cross over under the chin. This is used where the animal is hostile.

7.1.8 Pain and stress recognition:

Rats are exploratory animals in any new setting, Error in standing erect and an apparent lack of interest in surroundings is suggestive of ill health, though they are mainly non aggressive they exhibit considerable aggression and refusal to be handled in the face of repeated stress procedures, acute pain or discomfort produces vocalisation and struggles. They show tendency to lick or guard a painful area, they sit crouched and sleep-wake cycle will be abnormal if pain. The Harderian gland is a compound gland derived from the lacrimal gland, located on the bulbar conjunctiva of the third eyelid, which secretes a porphyrin lubrication which under normal circumstances is secreted to coat the eye. When the rat is stressed this secretion overflows on the face giving a stressing ring round the eye which denotes stress. This is known as chromodacryorrhoea. Red staining also may be observed at the nose of the patients since the fluid runs through the nasolacrimal duct.

7.1.9 Biological data and useful reference data

Biological data

Adult weight (g)	:	Male 450–520	Female 250–300
Diploid number	:	42	
Food intake	:	5-10 g/100 g bodyweight	
Water intake	:	10ml/100 g bodyweight	
Natural lifespan	:	3 – 4 (years)	
Rectal temperature (°C):		36- 40	
Heart rate/min	:	250-450	
Blood pressure (mmHg)	:	Systole 84-134	Diastole 60
Blood volume (ml/kg)	:	54-70	
Respiratory rate/min	:	70-115	
Tidal volume (ml)	:	0.6-2	

7.1.10 Drug doses for anaesthesia in rats

Medetomidine + ketamine:

Injected i.p at dose rate of 0.5 mg/kg of medetomidine 60-75mg/kg of ketamine. For small rats medetomidine may be diluted 1:10 with sterile water and the ketamine was mixed in the same syringe. Surgical anesthesia takes up to 30 min and wears off in 30 minutes after surgery. For recovery in 5-10 min reverse with atipamezole 1 mg/kg s.c.

Propofol:

Administered intravenously at 10 mg/kg propofol is used for brief surgical procedures or for the induction of anesthesia in cases where inhalation is to follow immediately.

Barbiturates:

Pentobarbitone is narrow safety margin and it should only be administered in terminal procedure. When given at a dose of 45 mg/kg i.p. it will cause anaesthesia that ranges from 15 minutes to 1 hour. But it will significantly lower the respiratory rate and have a high mortality index. Thiopentone can be administered I.V at 30 milles equivalent to Kgs.

Inhaled agents:

The preferred drug for this is isoflurane. It is useful for induction with an anaesthetic chamber and may be given for maintenance with a face mask.

Other drugs:

Doxapram may be administered at 5–10 mg/kg s.c., i.v. or i.p: however, in cases of respiratory depression this should be at a rate of 1–2mg/kg/minute, followed by a continuous infusion at half this rate to reverse the effects of morphine Atropine reduces salivary and bronchial secretion and may be given at 0.05 mg/kg.

7.1.11 Drug doses for analgesia in rats

Opioids

● Buprenorphine: 0.1–0.25 mg/kg s.c. or orally given 8-12 hourly. It is a partial agonist so blocks opioids like fentanyl but

does not counter the analgesic impact. It has slow onset of about 40 min so must be given before the animal regains consciousness and feels pain it but has a critical variable duration of action.

- Butorphanol: Use 0.5 to 2.0 mg/kg of Subcutaneous for 4 hours analgesia.
- Codeine: 50 or 60 mg/kg s.c. to give adequate analgesia for approximately 4 h.
- Morphine: Depend on the indication but in general, give 2–5 mg/kg s.c. for 2–4-h analgesia.
- Nalbuphine: Anidoprofen: give 1–2 mg/kg subcutaneously for analgesia of up to 3 hours.
- Pentazocine: Use it subcutaneously at 10 mg/kg for 3-4h operatory analgesia.

NSAIDs

- Carprofen: S.c injection of 2–5 mg/kg provides moderate to mild pain relief for 24 h. For oral administration dissolve 50 mg in 1250 ml water and give as much as will be consumed. Thus for a rat of 600 g drinking 60 ml water a day this will mean a daily intake of 4 mg kg. The made-up solution is stable for at least 24 h.
- Diclofenac: 10 mg/kg orally.
- Flunixin: 2.5 mg/kg s.c. lasts 12 h.
- Phenylbutazone: 20 mg/kg orally.
- Ibuprofen: 15 mg/kg orally lasts 4 h. [20]

7.2. MOUSE

Among all the lab animal, the mouse, Mus musculus is the most frequently used in the laboratories. A large number of well defined inbred strains and out-bred stocks are available and the karyo-types of these animals are also known. Notably, far more is understood about the genome of the mouse than of any other organism/for this reason among other the mouse is the most prevalently used experimental animal.

Figure 2 : Mus Musculus mouse

7 2.1 Behaviour

Mice are social animals they can live collectively in one cage with one male and many females, if only they define their role. It is model that shows how mice use pheromone in communication and one has to look into consideration when controlling the mice. The endocrine communication maintain order in the colony and if they are washed out every time the cage is cleaned, the mice will fight and the submissives, their fur may be groomed or they may be injured. Material enrichment to give them shelter, and the practice of putting a small portion of soiled litter into the new cage to minimize the constant resetting of the pecking order and scent posting will help to eliminate this.

7.2.2 Housing

Mice can be housed in standard cages where the pathogen status may be uncertain, but are more commonly maintained in barrier housing of controlled and well defined health status. Cages are often of the shoebox type made with polycarbonate plastic. Some of the requirements related to environment requirements included that bedding must be offered like wood chipping or commercially made paper based bedding. Males over the age of one begin to fight and may be kept individually. Female mice are least aggressive and therefore can be grouped together especially with familiar females

7.2.3 Feeding

This study were fed ad lib with a complete pelleted mouse diet; from hoppers placed above the floor to minimize contamination with feces. In general, mice eat 3–5g of pelleted diet daily but food intake varies with the stain, disease states, pregnant mice especially require more food than the normal mice.

7.2.4 Water

Mice require water for both purposes that is to facilitate them chew moistened food and for drinking but they cannot sustenance inadequate fluid stimulates difficulty in eating It is available in automatic dispensers or bottled.The normal dosage is 6 to 7 millilitres of water per day.

7.2.5 Environment

Another factor is that mice have a comparatively big amount of surface area to mass ratio and for this reason, get cold quickly and reacts considerably impressionably to temperature shifts. They spend a lot of effort regulating their body temperature and they cannot afford to have it lowered in the rooms. Another thing that the mice are vulnerable to is water loss. They cannot exactly pull off sweating or panting to let off heat since this imply dehydration. In the wild they utilize behavioral thermoregulation, for instance, burrow which cannot be employed using the conventional housing system in the laboratories. Hence, it becomes important to ensure that all conditions in the environment are well maintained. Mice should be maintained at temperature of between 19°C and 23°C, humidity of between 40% and 70%, airflow of 12-15changes per hour and 12 hours daylight daily. Light intensity should be about 350-400 lux, albinos excluded.

7.2.6 Growth

Some of the growth strains are fast growing while other are slowly growing at a faster rate. In overall, outbred stock grows much faster than inbred stain.

7.2.7 Handling

When handling small rodents it is preferable to lift off the lid of the cage than to open any flap that may exist on the lid before catching the animal. If overlooked they may crawl under the food hopper where they are almost impossible to catch and secondly they can be bitten. Mice move very fast, so you have to be quick and decisive to catch them:

1) Take the right hand, holding the base of the tail firmly with fingers and use the other hand to lift the mouse.

2) Place the thumb and the index finger of the other hand up the body with the other hand and grasp the scruff of the neck to control the head of the animal.

3) In this way, mice can be taken a short distance or shifted between cages It is best to place the mouse on a non-slip surface – such as the top of its cage or your arm – without letting go of its tail. The sex of the animal can then be determined by parting the tail and pulling down the external genitalia. The ano-genital distance in the male is of approximately double that observed in the female.

4) The animal is then immobilised and can be examined or have an injection made safely. The extra restraint may be obtained by pinching the tail with the fourth and fifth fingers of the hand.

7.2.8 Pain and stress recognition

Infrequent basal levels of activity It must be appreciated that there are probably genetic differences in various mouse strains with regard to certain stimuli but there are certain signals which are fairly clear; an animal will sleep more and lose weight if it wants to suggest to everyone that it has been through a procedure of some kind they will expect to cause it pain.

7.2.9 Biological data and useful reference data

Adult weight (g)	: Male 20–40	Female 18–40
Diploid number	: 40	
Food intake	: 15 g/100 g bodyweight	
Water intake	: 15 ml/100 g bodyweight	
Natural lifespan	: 1.5–3 years	
Rectal temperature (°C)	: 38–39	
Heart rate/min	: 310–840	
Blood pressure (mmHg)	: Systole 133–160	Diastole 90–110
Blood volume (ml/kg)	: 60–75	
Respiratory rate/min	: 60–220	
Tidal volume (ml)	: 0.18	

7.2.10 Drug doses for anaesthesia in mice

Ketamine combinations:

For surgical anaesthesia ketamine at 75 mg/kg i.p. with medetomidine at 0.5–1.0 mg/kg i.p. will give 20–30 min of surgical anaesthesia, which does not resisted by the animal if atipamezole (1 mg/kg s.c. or i.p.) is not used as a reversal agent, otherwise will give 2–4 h sleep time, It may also be used with xylazine (10mg/kg) same to the previous one though atipamezole is not as specific to counter xylazine. Whereas ketamine at a dose of 150 mg/kg i.p. with diazepam at a dose of 5 mg/kg i.p., or acepromazine at a dose of 2.5 mg/kg i.p., causes light anesthesia for twenty minutes with sleep time of two hours.

Propofol:

Continue to be given intravenously at 26 mg/kg it can be useful for short-term anaesthesia or only for induction of inhaled anaesthesia.

Barbiturates:

Pentobarbitone hazard index is very low and the drug can only be recommended for terminal surgical procedures only and respiratory effects will be profound. In some strains 45 mg/kg i.p. will exert an inconsistent reaction with sleep time ranging from 50 to 250 minutes; thus, over- or underdose with this drug entails undesirable next actions.

Inhaled agents:

Isoflurane is the preferred drug, Isoflurane is the best induction it can be used with an anaesthetic chamber while the maintenance is given through a face mask.

7.2.11 Drug concentrations for pain relief in mice

Opioids

- Buprenorphine: 0.05–0.1 mg/kg s.c. or i.p. those given 8 hourly. As is a partial -agonist; so reverses opioids, including fentanyl, but retains analgesia. It has a slow onset of about 40 min so must be given before the animal regains consciousness and feels pain, but it has a relatively long duration of action:
- Butorphanol: For moderate to severe pain give 1–5 mg/kg s.c. For lasting 4 hours of analgesia.
- Codeine: Conduct dosage by 60-90 mg/kg through oral administration or 20 mg/kg through s.c. For 4 h of pain relief.
- Morphine: Administer 2–5 mg/kg s.c. for 2–4 h of pain relief

NSAIDs

- Carprofen: S.C. 2–4 mg/kg provides a lasting effect in moderate pain for 24 hours. Orally suspend 50 mg (large animal solution) in 2500 ml water and administer free access. For a 30 g mouse given 4.5 ml water a day, this will amount to 4 mg/kg per day. The made-up solution is stable for a period not less than 24 hours.
- Diclofenac: 8 mg/kg orally
- Ibuprofen: 30 mg/kg orally. [21]

7.3 HAMSTER

That is why hamsters are famous for the cheek pouches, where they accumulate food to stock in deep burrow when photoperiod is getting lower. There are various types of hamsters which are employed and the by far the most prevalent is the Golden or Syrian hamster Mesocricetus auratus. There are strains for that too.

Figure 3 Syrian hamster

7.3.1 Behaviour

It might be added that hamsters make good pets because they are easily tamed and are not likely to nib at their masters; they occupy themselves with their teeth only if provoked or imprisoned. It finds men to be more submissive than women. They are daily active but predominantly night animals, therefore most activities are observed at night. Hamsters are territorial animals, they will fight each other.

7.3.2 Housing

Hamsters are wild animals, and as such, they like to be kept alone hence, each hamster should be housed separately. They are also good chewers so the plastic cages they use are those that have a rigid bottom.

7.3.3 Feeding

Unfortunately, information on the nourishment of hamsters is still scarce. They are coprophagic, but the vermin may have different digestive systems from rats or mice. People have suggested that it is enough to feed them a diet that has 16% protein, and 5-7% fat, and 60-65% carbohydrate. Hamsters feed on 5–7 g pelleted diet daily. Because they have blunt noses, they cannot be fed from standard wire mesh suspended hoppers. He needs hoppers with slot sizes bigger than 11mm so that they can use a scoop to pull the food through on the floor.

7.3.4 Water

These require approximately 10 millilitres of water per one hundred grams of the hamster's weight per day. This can be achieved by using water bottles or having automatic dispensing systems, but the sipper tubes themselves, must be made of stainless steel rather than glass because a hamster is quite capable of breaking the glass by biting it. Female are in a higher need of water, especially those who are breastfeeding.

7.3.5 Environment

Hamsters are originally originated in hot areas and they dig a cavity to prevent heat. If they cannot dig, they cannot stand heat at all. Cold, however, may be endured quite well. If the temperature gets below 5°C, they can fall into pseudodormancy, and if stimulated, they can come out of it. When the food component is low, it results that hibernation is not carried out to the full. The hamsters should be housed at a temperature of 19 to 231C and the breeding hamsters require the higher temperatures. As for relative humidity it should be 40 to 60 percent. Light is here advised to be provided at a period of between 12 to 14 hours.

7.3.6 Handling

Its characteristic feature is the desire to bite if the hamster is frightened, so this animal should be taken by pinching it in the palm of the hand. These small rodents have no tail and also have big cheek pockets within their faces. They are restricted by holding a big handful of their scruff and rolling over onto the hand as is done to mice. If only minimal amount of scruff is pulled, the hamster moves around and bites. Further restraint may be obtained by encircling skin on back with each finger and encircling palm as well.

7.3.7 Pain and stress recognition

Signs that hamsters will exhibit are loss of weight, longer sleep cycle and aggression or depression. Ocular discharge is also followed by diarrhoea and may be due to signs of stress.

7.3.8 Biological data and useful reference data

Adult weight (g) : Male 85–130 Female 95–150
Diploid number : 44
Food intake : 5 g/100 g bodyweight
Water intake : 10 ml/100 g bodyweight
Natural lifespan (years) : 1–3
Rectal temperature (°C) : 37–38
Heart rate/min : 250–500
Blood pressure (mmHg) : Systole 150 Diastole 100
Blood volume (ml/kg) : 78
Respiratory rate/min : 35–135
Tidal volume (ml) : 0.6–1.4

7.3.9 Drugs doses for anaesthesia in hamster

Fentanyl/fluanisone combinations

In cases of surgical procedures, Hypnorm at 0.5-1 ml/kg i.p. should be administered in combination with midazolam of 5mg/kg. Understand that absorption from the peritoneal cavity may be unpredictable but the anaesthesia should last about 20-40 minutes.

Ketamine combination

Ketamine in the dose rate of 200mg/kg + xylazine 10mg/kg i.p gives rise to surgical anesthesia though there will be moderate respiratory embarrassment. Ketamine 150 mg/kg i.m. can
be administered with acepromazine at 5mg/kg i.m.

Propofol

Propofol can be used at 10 mg/kg i.v.

Barbiturates

Using a dose rate of 50-90 mg/kg pentobarbitone will induce anaesthesia but with high mortality since the security factor is almost nil. Thiopentone has a rapid acting intravenous dose that is preparatory at 30mg/kg.

Inhaled agents

Isoflurane is the best agent for preventing intraoperative awareness. For induction it can be used with an anaesthetic chamber and for maintenance the drug can be administered through face mask.

7.3.10 Drug doses for analgesia in hamster

- Buprenorphine 0.5 mg/kg s.c.
- Butorphanol 0.4 mg/kg s.c.
- Flunixin 2.5 mg/kg s.c.
- Pethidine 20 mg/kg s.c. [22]

7.4 GERBIL

More than a hundred species are found in this group, though most often the Mongolian gerbil, Meriones unguiculatus is used for experimentation. These are easy to keep.

Figure 4 Mongolian gerbil

7.4.1 Behaviour

Gerbils are comparatively gentle animals, which do not disturb when held in hands, and almost never use their teeth. Usually they are very lively, and if they are chased, they will not want to get caught. They tend to display investigative behavior in unfamiliar territory and when those are free they do not seek a nearby place to lay focusing on the surrounding environment. Wild ones are of crepuscual nature. While friendly with people, the unfamiliar adult, the caged together will be aggressive.

7.4.2 Housing

As with the majority of rodents, gerbils require specific husbandry conditions to feel comfortable and be healthy. They prefer solid floors to mesh, and require a depth of 2cm of bedding for nest building which takes place whether or not the female is pregnant. Gerbils should be given at least 15cm space between top of the bedding and the roof of their cage since they prefer to stand and sit. Since gerbils like to bite the cage they should be made of a sufficiently sturdy material for it not to be an easy task to chew through it in an attempt to escape. Gerbils produce very small amounts of urine and small hard round faecal pellets. These animals are inherently clean and as such the cages only requires washing about once a week normally.

7.4.3 Feeding

Also like all rodents, gerbils are coprophagic. Consuming extends all through the day and up to night time. Standard diets containing 22% protein are sufficient for the rat, but fibrate altered lipid metabolism is such that no more than 4% dietary fat should be attributed to the development of hyper cholesterol. The levels of fat result in obesity, and in females, hurt fertility because fats accumulate in the genital tract. Pellets can also be soaked. Gerbils require 5 to 8 gm of the preformed pelleted food per day.

7.4.4 Water

Gerbils by their nature will survive on very little water but on the down side older males require more water than younger gerbils. In fact, they pass very concentrated urine, and do not limit their ability to prevent further water loss. Gerbils need about 4-7 ml of water per day.

7.4.5 Environment

The gerbil can thus endure hot temperatures and control their body heat well because gerbils originate from desert areas. Glasshouse fungi are optimally grown under conditions ranging from 19°C to 23°C. Relative humidity should remain between 30 to 50 % and 12 h light ranging at 350-400 lux should be administered daily. Air change should provide a rate that should not be less than 12 and not more than 15 changes per hour.

7.4.6 Handling

It is similar to mouse and rat to restrain gerbils using some of following mentioned methods. They may be picked up by taking the base of the tail in one hand and holding the body by the other hand.. A lot of delicate should be used in handling because if you hold the shaft of the tail the skin will slip off.

7.4.7 Pains and stresses identification

Gerbils are friends or substrates, will lose weight and may develop piloerection and a scruffy coat whilst also becoming more aggressive or depressed. It was also noted that stress might cause both ocular discharge and diarrhoea. Facial markings are identified in the gerbil.

7.4.8 Biological data and useful reference data

Adult weight (g) : Male 65–100 Female 55–85
Diploid number : 44
Food intake : 5–8 g
Water intake : 4–7 ml
Natural lifespan (years) : 3–4
Rectal temperature (°C) : 37–38.
Heart rate/min : 360
Blood volume (ml/kg) : 66–78
Respiratory rate/min : 90

7.4.9 Anaesthetic drugs dosage and the gerbil

Fentanyl/fluanisone combinations:

In surgical cases Hypnorm is given at 0.5- 1 ml/kg i.p with water in conjunction with midazolam at 5 mg/kg i.p. The Hypnorm may be reversed with naloxone 0.05 mg/kg or for further analgesia with nalbuphine 4.0 mg/kg i.p. or s.c., butorphanol 2.0 mg/kg i.p. or buprenorphine 0.1 mg/kg s.c.

Ketamine combinations:

Ketamine 100 mg/kg with xylazine 2 mg i.p. causes moderate to deep commercial anaesthesia in gerbils but it is an unreliable surgical anaesthesia and it does superficial respiratory paralysis. Alternatively, ketamine at 50 mg/kg in i.m route with diazepam five mg/kg in IP route.

Barbiturates:

An intravenous dose rate of 60–80 mg/ kg pentobarbitone will attain anaesthesia but at the cost of a high mortality rate because the safety factor here is low.

Inhaled agents:

It pointed out that isoflurane is the preferable agent. For induction it can be used in an anaesthetic chamber and for maintenance through a face mask.

Other drugs:

It is also advisable to also administer atropine 0.04 mg/kg s.c., when the animal is asleep to prevent accumulation of secretions to the extent that they block airways. To manage respiratory depression, use doxapram 5 – 10 mg/kg.

7.4.10 Drug doses for analgesia in gerbil

- Buprenorphine, 0.1– 0.2 mg/kg s.c.
- Butorphanol, 2.0 mg/ kg i.p. or s.c
- Pethidine 20 mg/kg subcutaneously.[23]

7.5 ZEBRAFISH

It is now increasingly used in genetics, pharmacology and behavioral studies. Like any other vertebrates, the zebrafish have a similar genetic map to humans and are also being adopted as models for human diseases. Zebrafish has certain advantages compared to other popular vertebrate models, such as high ratios of fecundity, relatively low cost of maintenance, embryos being transparent and short generation time.

Figure 5 Zebrafish

7.5.1 Behaviour

1. Locomotor Activity:

Morphological alterations as well as changes in the swimming patterns, speed, and exploratory behavior of the zebrafish animals are monitored. Changes in either the amount or frequency of patient movement can signify variations in neurological and physiological activity such as stress or anxiety.

2. Anxiety-Related Behavior:

To evaluate anxiety-like behaviour protruding to the novel tank diving test is ubiquitous in anxiety test paradigms used to study zebrafish. Normal zebrafish when introduced to a novel tank first plunge to the bottom and gradually swim in higher areas of the tank, and continuous hovering at the bottom implies anxiety.

3. Social Behavior:

Zebrafish is also a social fish, where it is possible to examine social preference or avoidance towards them. Shoaling, that is group swimming is reported to assess social integration and disturbances in the abilities are likely to imply social dysfunction or stress.

4. Aggression and Predatory Behavior

Certain works concern the aggression of zebrafish and might use reflection tests or, on the contrary, social conditions. In this case, aggression may be determined by something like changes in the environment or exposure to chemicals.

5. Stress Response:

Stress hormones cortisol and behaviour during stress-challenge situations (e.g., exposure to predation or novelty) are assessed. These responses are used in order to evaluate how stress affects the nervous system and the behavior of the individual. [24]

7.5.2 Housing

1. Tank Setup:

Usually, zebrafish are kept in fish bowls with mature water which circulates to allow for water changes with minimal disturbance to the water conditions by the fish.

There is always a filter and an heater, and occasionally a UV sterilizer to minimize contamination of the tank.

2. Water Quality:

Water parameters are critical and include maintaining:

pH: 6.8 to 7.5

Temperature: 26-28°C

Conductivity: 300-1500 µS/cm

Supplementing feeding plays a role in monitoring of ammonia, nitrite and nitrate level where ammonia levels should remain as close to zero as possible. The water should closely resemble that found in nature and the water should also be changed from time to time in other to avoid the build up of waste products and various dangerous chemicals.

3. Lighting and Photoperiod:

As a rule, 14/10 or 10/14 light-dark cycle is sustained to simulate the natural day and night periods. The possibility of building lighting should be optimal to ensure the animals are treated naturally without straining them with very bright lights.

4. Density and Group Housing:

Zebrafish is a social fish and should be grouped together. One rule of thumb is the density factor, which is usually in the range of 5-10 nationally liter water and regarded depending on experimental requirements and strained aquaculture systems. [25]

7.5.3 Feeding

Zebrafish should not be fed so often because it will lead to the accumulation of nitrates in the water hence may have an effect on breeding or viability because some of the fish may die from this but if fed adequately they will be so aggressive. We suggest serving in any feeding time, no more food that what a tank of fish is able to consume within 10 minutes. This is because concentration of salt has a deleterious effect on the zebrafish hence it is very crucial to avoiding feeding the shrimps to the zebrafish wit salt. To produce more zebrafish eggs, the fish can be fed thrice daily. Additionally, performing daily water changes on breeder fish tanks have the added benefit increasing the levels of egg production. [26]

7.5.4 Water:

Water Source: This water may be tap water which has been then filtered and dechlorinated or water with reverse osmosis or purified water whose characteristics has been altered artificially to fit natural standard.

pH: According to ZebSource, the general pH range of the water used for zebrafish is 6.8 – 7.5.

Temperature: Zebrafishes are tropical fish their optimal temperature is between 26-28°C.

Dissolved Oxygen: There must always be enough aeration so that must be at least 6 mg/L or higher.

Water Changes: To discuss the needs of the fish as far as the water is concerned, it is clear that an aggressive procedure is followed whereby waters are exchanged frequently, at least 10-20% per week to enhance water quality. [27]

7.5.5 Environment

Tank System: Zebrafish are generally kept in a recirculating water system with transparent elements such as the material used for construction of the tank (acrylic). It is found that many tanks are grouped in racks based on their sizes that may vary depending on the number of fishes that are put in the tanks.

Filtration: Recirculating systems usually use mechanical, biological and chemical filteration mechanisms such as use of activated carbon to enhance water quality. UV sterilization is commonly used in cleaning area and passages in order to eliminate bacterial or viral threats.

Behavioral Enrichment: While not all laboratories highlight the importance of enrichment for zebrafish (like hiding places, plants etc.), it has now been accepted that it is good for their health. [28]

7.5.6 Growth

Growth Stages of Zebrafish:

Embryonic Stage: The zebrafish embryos mostly hatch at 2–3 days post fertilization at 28°C. Five days post feeding, the larvae are developed and become mobile, actively swimming and feeding endogenous (off the environment).

Larval Stage: There is significant growth during this stage. Larvae normally are 3.5 – 4.5mm at hatching and reach about 10 – 12mm during late larval stage.

Juvenile Stage: Juveniles continue to grow in length and weight and are typically 2-3 cm by 90 days post fertilization. Developmental changes continue and sexually distinction starts as male and female can be distinguished. [29]

7.5.7 Handling

Zebrafish used in laboratories require proper anaesthesia and analgesia during handling and this forms part of the animal welfare.

Handling During Anesthesia:

Induction: Zebrafish are calmly scooped with a net or tipsed over into another container, which has anesthetic solution. Physical contacts must be avoided as much as possible since the zebrafish is known to be sensitive to stress and dibles. Small portion training reduces interference to the nurses and therefore facilitates a smooth flow on induction.

Maintenance of anaesthesia: For operations that may take long, such as surgery, the fish can be kept in the anesthetic solution or can be kept on a wet surface while water containing the anesthetic agent is dripped on the gills to keep the fish anesthetized. [30]

Analgesia in Zebrafish:

Lidocaine: Applied locally using external gel or as subcutaneous injection lidocaine serves as Local anaesthetic in zebrafish.

Opioids and NSAIDs: While not commonly applied for zebrafish, opioids, including morphine, and NSAIDs Meloxicam have been investigated for the purpose of analgesia.

7.5.8 Pain and stress recognition

It is known that zebrafish can respond to the pain similarly to mammals do not, but there are objective signs that allow the assessment of its condition.

Indicators of Pain and Stress in Zebrafish:

1. Erratic Swimming: Any sign of stress include frequent swimming, abnormal movements, or attempts to run away etc.

2. Reduced Activity: On the other hand referring to the degree of mobility, cold or lack of it may point towards pain or otherwise discomfort.

3. Hiding: Chinook salmon that the researchers exposed to stress seemed to move away or stay out of sight and this may be interpreted as the fish perceiving threats.

4. Body Posture: Belly crawling or twisting in a way that is not typical includes swimming sideways which may indicate discomfort**.** [31]

7.5.9 Biological data and useful reference data:

Adult length	: male -23.1mm	female-24.9mm
Weight	: male -0 23gm	female-0.36gm
Diploid no	: 25 pairs	
Lifespan	: 3-5 years	
Heart rate	: 162 beats per minute	
Temperature (F)	: 70-90 F	

7.5.10 Drugs of doses for anaesthesia in zebrafish

Common anesthetic agents and dosage used for Zebrafish

1. Tricaine Methanesulfonate (MS-222):

Dosage: Anesthesia – 100 to 300 mg/L immersion.

pH Adjustment: Septic pericarditis might be present and the pH should be buffered to 7.0-7.5 with sodium bicarbonate.

Induction Time: Normally between 1 and 2 minutes at a concentration of 150 mgCL- Depending on the size and health of the fish.

2. Eugenol (Clove Oil):

Dosage: 40 to 100 mg/L for immersion.

Considerations: Ethanol particularly should be added to clove oil because it acts as a solvent that emulsifies the oil in water.

3. 2-Phenoxyethanol:

Dosage: 200 to 400 mg/L for immersion of the samples in natural sea water.

Induction Time: Depends on the concentration but it starts functioning in a few minutes.

4. Lidocaine:

Dosage: 1-2% (10,000 to 20,000 mg/L) as a topical anesthetic in operative and diagnostic minor procedures. Local application is used in surgery and can be administered through injections as well.

Considerations for Anesthesia

Monitoring Depth: To even gauge the amount of anesthesia needed it is important to assess the fish for signs that it has lost balance and also its level of response to the stimuli.

Recovery: After the procedure fish should be taken to newly well aerated water to recover. The recovery time will depend on which anesthetic was applied, as well as the levels of concentration in the solution.

Stress Indicators: Behavioural changes before and after the anesthesia can also be observed before coming to a general conclusion about the welfare of the fish. [32]

7.5.11 Drugs doses for analgesia in zebrafish

1. Meloxicam:

Intraperitoneal Injection: 5 mg/kg body weight (0.5 mL per fish) can be used before or after the various procedures.

Duration: Usually side effects are felt for not more than one day after administration has taken place.

2. Aspirin (Acetylsalicylic Acid):

Oral Administration: Up to 24 hours before the procedure must use water with an acclimatization of 100 mg/L.

Consideration: Prolonged treatment in most cases may be required to achieve the required level of pain relief.

3. Buprenorphine:

Subcutaneous Injection: 0.1-0.5 mg/kg body weight applied that is useful for pain control after the operation.

Duration: Gives a few hours of pain relief on affected areas.

4. Gabapentin:

Oral Administration: 10-30 mg/kg body weight in food or water: such as Prostaglandin E2 (PGE2), Indomethacin sodium, Pheneturin sulphate, Flumioxazin. [33]

8. CONCLUSION:

In conclusion, the use of anesthesia and analgesia in laboratory animals is essential to ensure humane treatment and ethical research practices. Correct administration minimizes pain, discomfort and physiological stress, thus improving animal welfare and the reliability of experimental results. Careful selection of anesthetic and analgesic agents, dose adjustment based on species-specific requirements, and close monitoring are essential to minimize potential risks and complications. Compliance with regulatory guidelines and ethical standards ensures that animal research is conducted responsibly, balancing scientific objectives with the imperative to reduce animal suffering.

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