CHAPTER 5

ANIMAL WELFARE CONSIDERATIONS

CHAPTER 5.1

SPECIES SPECIFIC CAGING CONFIGURATION AND DESIGN

Cindy Phua

To minimize experimental variables, laboratory animals should be housed in caging systems that fulfil certain minimum requirements. These requirements can be classified into two categories, those that benefit the users and those that benefit the animals. The macroenvironment and facility design benefit both, while the microenvironment is of primary benefit to the animals.

Temperature, humidity, ventilation, light intensity and duration, noise and other variables contribute to the macroenvironment that directly affects the animal's cage environment, known as the microenvironment. The macroenvironment and microenvironment need to complement the facility design to ensure an optimum environment for the users and the animals.

There are three broad categories of facility design. They are barrier, containment and conventional facilities. A barrier facility is designed to keep out contamination whereas a containment facility designed to keep contamination from leaving the facility. Conventional facilities (often containing animals of mixed health status) may not have definitive design specification for "clean" to "dirty" traffic flow. Different types of animal will require different barrier needs, for example, specific pathogen free (SPF) animals may require a barrier facility for a more stringent barrier to prevent cross-contamination from conventional facilities, whereas animals subjected to infectious agents will need a containment facility to to hinder the release of infectious contaminants.

Caging Systems

Caging system can be open or isolated. Open caging systems have a direct interaction with the macroenvironment. In open systems such as the metal rack caging and animal pens, animals are subjected to the variables of the room environment and users are also exposed to agents and allergens associated with the animals housed. These caging systems are common in barrier and conventional facility for large animals such as the rabbits and nonhuman primates.

Isolated caging systems are commonly used for small animals, like rodents, and when a high level of barrier and containment is required; examples of these static micro-isolators and individually ventilated cages (IVCs) where an external fan unit ventilates the system. The fan unit supplies HEPA-filtered air to the cages at a high air exchange rate. While the older IVC systems only provided supply source, newer models have been developed to provide a controlled supply and exhaust function. This allows animal facilities to control the air pressure and the number of air changes in the cages. The air supply and exhaust can be adjusted for positive or negative pressure air balancing of the cage relative to the room. If set at negative, filters on the cage top keep contaminants out and thus, provide a clean environment for the animals. If the air balancing is set to make the cage positive to the room, the cage filter-top ensures that circulated air is filtered before it is released to the room and acts as a barrier for the cage if the fan unit fails. The filter-top also protects the animal when the cage is removed from the IVC rack. Exhausted air can be hard-ducted out of the room to reduce rodent odours. In high-level containment facilities and the exhausted air can be filtered before being released to the outside of the facility.

Cage Design

Cage design is dependent on the age, weight and size of the species. It should be escape-proof and made from caging material that is sturdy, durable and have a smooth and impervious surface for easy sanitation. The cage should also allow monitoring without disturbing the animal. The feeding tray and water source should be easily accessible by the animal and also be easily cleaned and disinfected. Fig 5.1.1 shows one such cages for rabbits.

Animal caging should provide sufficient space in accordance to the weight range of the species and the number of animal housed per cage. Animal facilities must comply with the recommended cage size listed in the "*Guidelines on Care and Use of Animals for Scientific Purposes*" by the National Advisory Committee for Laboratory Animal Research (NACLAR). Researchers who breach the guidelines will be reported to the Institutional Animal Care and Use Committees (IACUC) and may have their projects suspended.

Due to its strength, durability and the ability to withstand repeated sanitation, stainless steel is a common material used. This material is more cost-efficient as the need to replace cages will be reduced. The strength of stainless steel makes it suitable for housing large animal due to their weight. Moreover, stainless steel does not corrode with most disinfectants and can withstand repeated high temperature washing.

Open rack caging can be made fully or partially from metal, usually stainless steel. They consist of perforated flooring that allows waste to be collected into a tray. Open rack caging can be modified to suit the user's and the animal's needs. For example, nonhuman primate cages (Fig 5.1.2) are equipped with a perch and an additional back panel that can be moved, called the squeeze-back. The squeeze-back mechanism moves the animal to the front of the cage to partially immobilize the animal for manipulation through the cage front or for injections. Another example of the open system is the animal pens, commonly used to house larger animals such as the swine and the sheep. Animal pens consist of a stainless steel framework and a non-slip impervious flooring that is easily disinfected and usually equipped with a good drainage system.

While IVC racks are generally made from stainless steel, the individual cages are usually made from plastic polymers. The types of plastic polymers range from the most basic polystyrene to thermosplastic such as polysulfone and polyetherimide. Polystyrene cages cannot endure repeated high temperature washing and autoclaving but can be disinfected. They are frequently used in research where routine decontamination is not feasible, such as research where radioactive and hazardous agents are used. Polycarbonate cages are commonly used, but the lifespan of use is shorted by frequent autoclaving. Polysulfone and polyetherimide cages have a longer lifespan and can withstand high temperatures of 150°C and 160°C respectively. These thermoplastic cages can be autoclaved to house SPF and immunodeficient animals.

Fig 5.1.1: Rabbit cage.

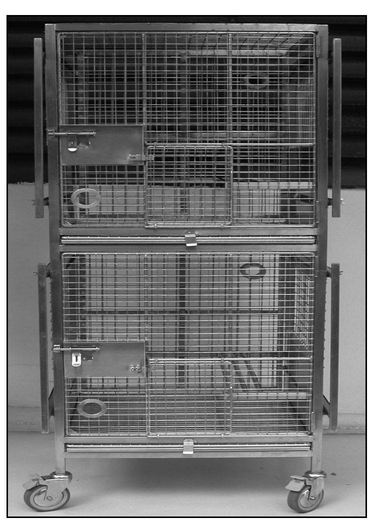

Fig 5.1.2: Meshed cage – monkey.

Cage Accessories

Cage enrichments are required for almost all species of animals especially social animals and individually housed animals. These provide stimuli that encourage the expression of species-typical behaviour. Individual housing can be traumatic to the animals and enrichment can reduce this stress.

Enrichment accessories such as disposable paper roll and polymer vinyl chloride (PVC) cylinder (Fig 5.1.3) provide shelter and gnawing for the rodents. Running wheel also provide enrichment for rodents and may be encouraged for breeding colonies where overweight breeders are discouraged.

Enrichment toys such as a plastic chain or plastic ball are commonly used for most large animals for gnawing and visual stimuli, such as those for pigs (Figs 5.1.4 and 5.1.5) and rabbits (Fig 5.1.6). Heavy plastic is often used as it is durable, economical and can be easily disinfected.

Nonhuman primates require a more intensive enrichment programme due to their complex needs. Group or pair housing should be provided whenever possible for social enrichment. Visual, auditory or olfactory contact with other primates should be provided if animals must be individually housed. In some cases, grooming contact can be provided while keeping animals in individual cages. Nonhuman primates should be provided with a perch, manipulanda such as toys and foraging devices or opportunities. Over-grooming commonly associated with individually housed animals can sometimes be reduced by providing complex, time consuming foraging opportunities. Mirrors or reflective toys will also help provide visual enrichment. Fruit and/or vegetable treats can also be apart of an enrichment programme, but should not be allowed to replace the balance ration. If using outdoor pens for nonhuman primates the weather should be taken into consideration. Rain cover, shade and protection from temperature extremes should also be provided.

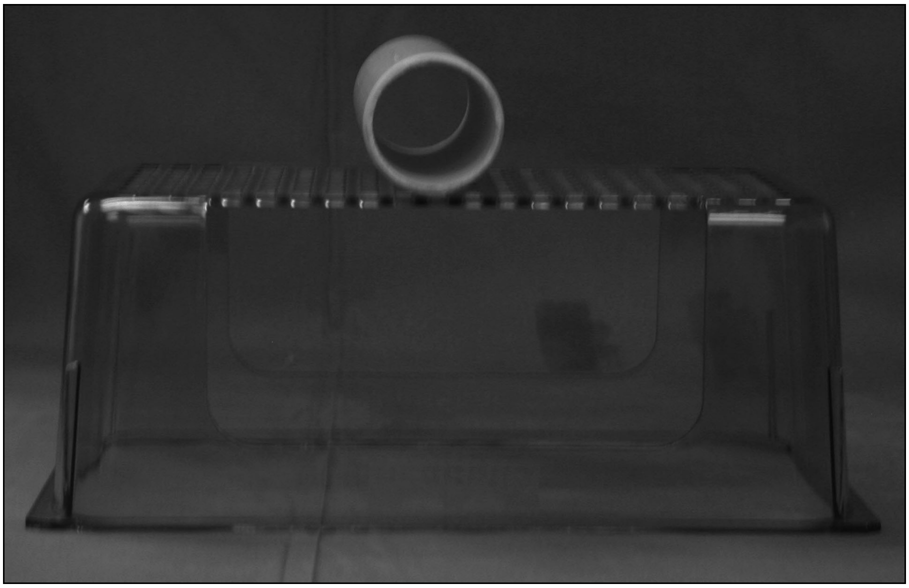

Fig 5.1.3: Enrichment for rodents.

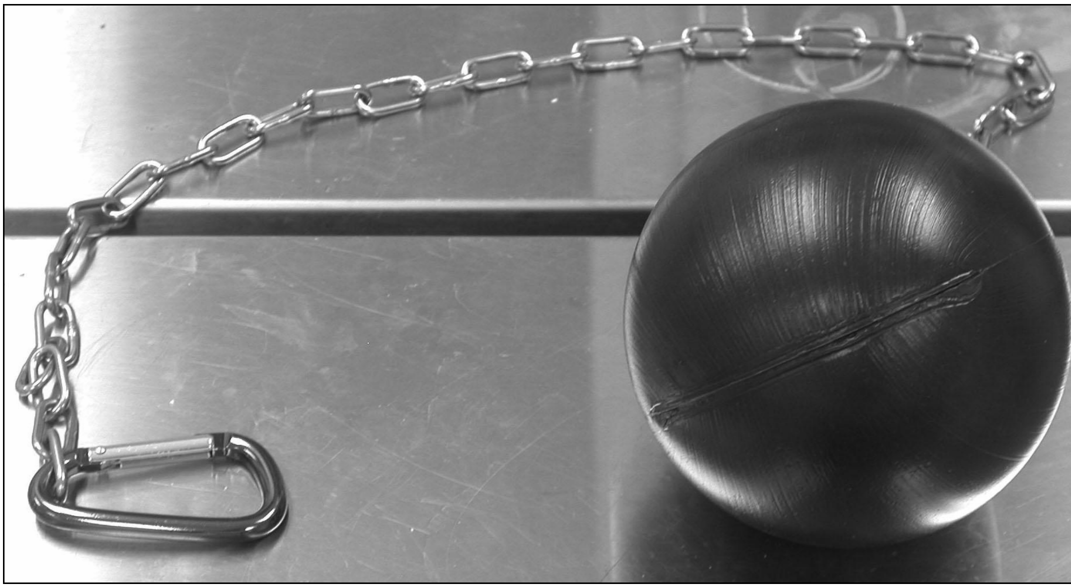

Fig 5.1.4: Enrichment I for pigs.

Fig 5.1.5: Enrichment II for pigs.

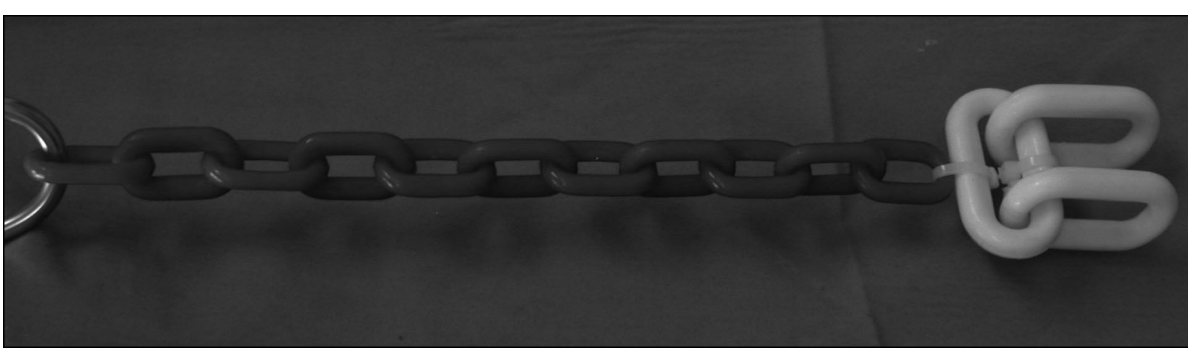

Fig 5.1.6: Enrichment for rabbits.

Other Caging Systems

1. *Transport Cages*
   Transport cages provide mobility and temporary housing for animals. These cages should have adequate room for the animals, be escape-proof and well ventilated. While transport cages provide temporary housing, for example when the animal cages need to be sanitized, the animals should not be housed in them for prolonged periods.

2. *Recovery Cages*
   A recovery cages or intensive care unit (Fig 5.1.7) are used in post surgical recovery and for clinical care of sick animals. They are usually mobile and constructed of fibreglass. The units are also fitted with environmental controls that regulate humidity, temperature and oxygen levels.

Fig 5.1.7: Intensive care unit/recovery cage.

3. *Restrainers*
   Restraining devices are designed to immobilize animals for short-term handling while they are being treated or manipulated. Specific restraint device depends on the species being restraint. Plastic rodent cyclinder (Fig 5.1.8), rabbit restraint box (Fig 5.1.9), pig slings and nonhuman primate chairs (Fig 5.1.10) are examples of restrainers.

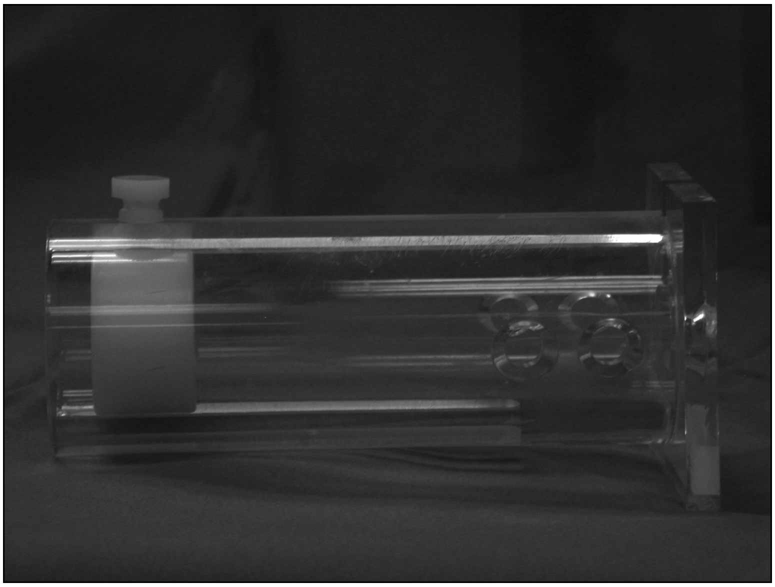

Fig 5.1.8: Rodent restrainer.

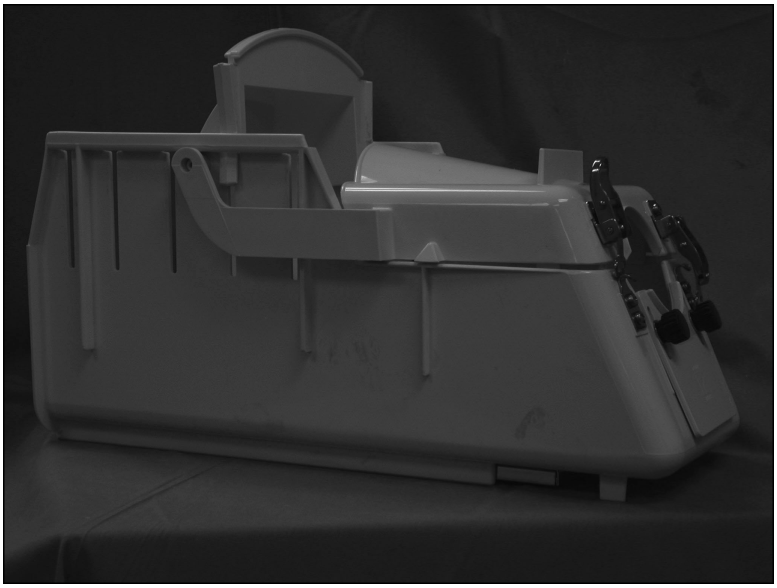

Fig 5.1.9: Rabbit restrainer.

Fig 5.1.10: Nonhuman primate restrainer.

Standards for Housing and Environmental Conditions

Tables 5.1.1, 5.1.2 and 5.1.3 list the standards practice for housing of different types of animals and environmental conditions.

Table 5.1.1: Goat, sheep and swine

Animal	Weight/kg	Floor area/m^2	Height/cm
Goat/Sheep	< 25	0.8/0.765/0.675	-
	Up to 50	1.35/1.125/1.017	
	> 50	1.8/1.53/1.35	
Swine	< 15	0.72/ - / -	-
	Up to 25	1.08/0.54/0.54	
	Up to 50	1.35/0.9/0.81	
	Up to 100	2.16/1.8/1.62	
	Up to 200	4.32/3.6/3.24	
	> 200	≥ 5.4/≥ 4.68/≥ 4.32	

Table 5.1.2: Rabbit, cat, dog, chicken, nonhuman primate

Animal	Weight/kg	Floor Area/m^2	Height/cm
Rabbit	< 2	0.135	35
	Up to 4	0.27	
	Up to 5.4	0.36	
	> 5.4	> 0.45	
Cat	≤ 4	0.27	60
	> 4	≥ 0.36	
Dog	< 15	0.72	-
	Up to 30	1.08	
	> 30	≥ 2.16	
Chicken	< 0.25	0.225	-
	Up to 0.5	0.045	
	Up to 1.5	0.09	
	Up to 3.0	0.18	
Monkey	Up to 1	0.144	50
	Up to 3	0.27	76
	Up to 10	0.387	

Table 5.1.3: Mouse, rat, hamster and guinea pigs

Animal	Weight/kg	Floor Area/m^2	Height/cm
Mouse	< 10	38	12
	Up to 15	51	
	Up to 25	77	
	> 25	> 96	
Rat	< 100	109	17
	Up to 200	148	
	Up to 300	187	
	Up to 400	258	
	Up to 500	387	
	> 500	< 451	
Hamster	< 60	64	15
	Up to 80	83	
	Up to 100	103	
	> 100	> 122	
Guinea Pig	≤ 350	387	17
	> 350	> 651	

* Larger animals might require more space to meet performance standards

CHAPTER 5.2

POSTOPERATIVE CARE AND PAIN MANAGEMENT

Jason Villano

Any manipulation of laboratory animals such as handling and physical restraint, surgical procedures and even routine procedures such as blood collection can have profound effects on their behaviour and physiology, which can variably be reflected in the research results. Inasmuch as unnecessary data or data misinterpretation are concerns in the quality of research derived from these animals, animal welfare posts a significant role in the conduct of these experiments, particularly those involving operative procedures.

The research personnel led by the principal investigator and the animal care staff led by the institutional veterinarian are the two groups that play key roles in developing an effectively managed postoperative care programme tailored to the institution's needs. The veterinarian and his staff can recommend and implement measures on alleviating the pain and distress of the animals while it is the researcher's responsibility to show that he has considered all these options without compromising the validity of the research. Effective communication between these two groups is thus essential.

Postoperative Support

The postoperative period can be divided into three phases, anaesthetic recovery, acute postoperative care, and long-term postoperative care.

1. *Anaesthetic Recovery*
   Frequent and careful observation is required during this stage as it is the most critical. Great physiologic disturbance and crises can arise quite rapidly at this time. Large

animals, whose trachea is intubated for inhalation anaesthesia during the surgery, can vomit and suffer from aspiration pneumonia. The animal should only be extubated when the gagging or swallowing reflexes have returned. The animal's vital signs, cardiovascular and respiratory functions must also be checked and maintained. Rotating or turning over the animal's body every 30 to 60 minutes until it has recovered from the anaesthesia will facilitate respiration and avoid dependent edema. Usually, animals should be individually housed during recovery in cages that have been sanitized between usage.

2. *Acute Postoperative Care*

During this stage, the animal is usually maintained in the recovery area such as an ICU (Fig 5.2.1) until adequate stabilization allows removal to a more standard husbandry situation (that is, eating and drinking have resumed and critical physiological parameters are within acceptable ranges for the model created). Pain management should be started at this time or continued if preemptive analgesia has been given. The investigator and/or the veterinary staff must be familiar with the animal's normal behaviour and posture considering species and individual variation. Unless there is evidence to the contrary, it has to be assumed that a procedure or a condition painful for humans will also be painful for animals.

Besides the analgesics, parenteral fluids and antibiotics may be continued. The hydration status should be monitored as overhydration results in frequent urination and pulmonary edema while underhydration results in sticky mucous membranes, loss of skin elasticity, the eyes sinking into the orbit, decrease in blood pressure and increase in heart rate. Replacement of blood loss is with saline or lactated ringers administered three times the volume of blood lost by slow intravenous drip. Monitor the hematocrit. If it drops below 20 %, whole blood replacement may be necessary.

Fig 5.2.1: Intensive care unit.

3. *Long-Term Postoperative Care*
   Although it is often the most neglected phase in the clinical monitoring, long-term management is equally important as it returns the animal to as normal a physiological and behavioural state as possible. This includes wound management, monitoring appetite, body weight and activity.

Wound management prevents infection and inflammation and facilitates the healing process. When drains, collars and dressings are used, the animal's ability to eat and drink should not be hampered. If the wound is exposed, daily cleaning and monitoring needs to be done to remove accumulated dirt such as faeces. Chlorhexidine or iodine swabs and antibiotic ointments or powder may be used (Fig 5.2.2). Conversely, if the wound is covered with a dressing, regular cleaning and changing should be done as often as every other day or as necessary when the dressing is wet. Any external sutures are removed once skin incision site is healed, usually between 10 to 14 days.

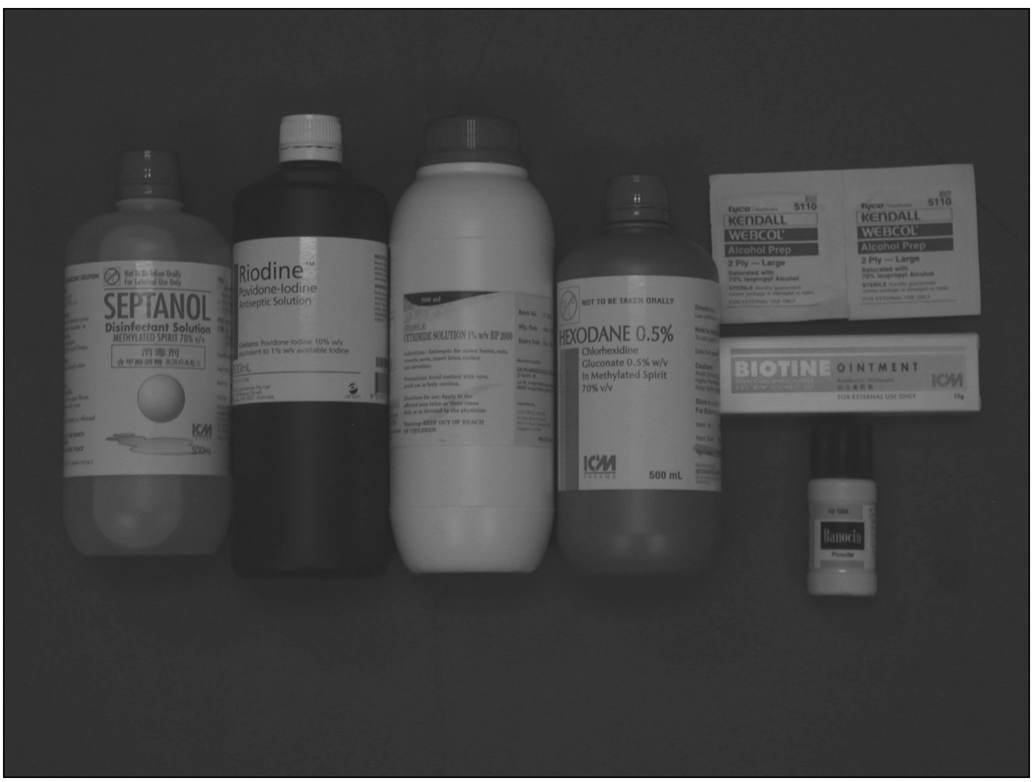

Fig 5.2.2: Commonly used disinfectant.

The quantity and quality of the faeces and urine should also be monitored because changes may indicate several postoperative complications such as paralytic ileus, renal shutdown or irritation hypermotility. Regular checks on the body weight and appearance and the animal's appetite should also be done. Physical therapy may also be needed in some cases for postoperative paresis or paralysis.

Pain Management

This is an important aspect in the perioperative care as it has profound effects on the animal's physiology and behaviour and it addresses the issues and concerns of animal welfare (Fig 5.2.3). An animal's response to pain is often adaptive to reduce movement minimizing re-injury and aiding recuperation. However, this response may lead to changes which impact negatively on both the animal's well-being and research results.

Examples of procedures that may cause pain or distress are physical restraints, survival surgeries, tumour burdens, intracardiac or orbital sinus blood sampling, and abnormal environmental conditions. These procedures can cause changes in the heart rate, blood pressure, respiration and body temperature. Blood glucocorticoid and catecholamine levels are also usually elevated.

Otherwise, it is difficult to assess pain and distress in animals because of their inability to communicate directly. Because of this, animal welfare regulations require that analgesia be provided whenever a procedure is to be performed or a condition is present that is likely to cause pain. It is best if analgesia can be provided to animals preemptively.

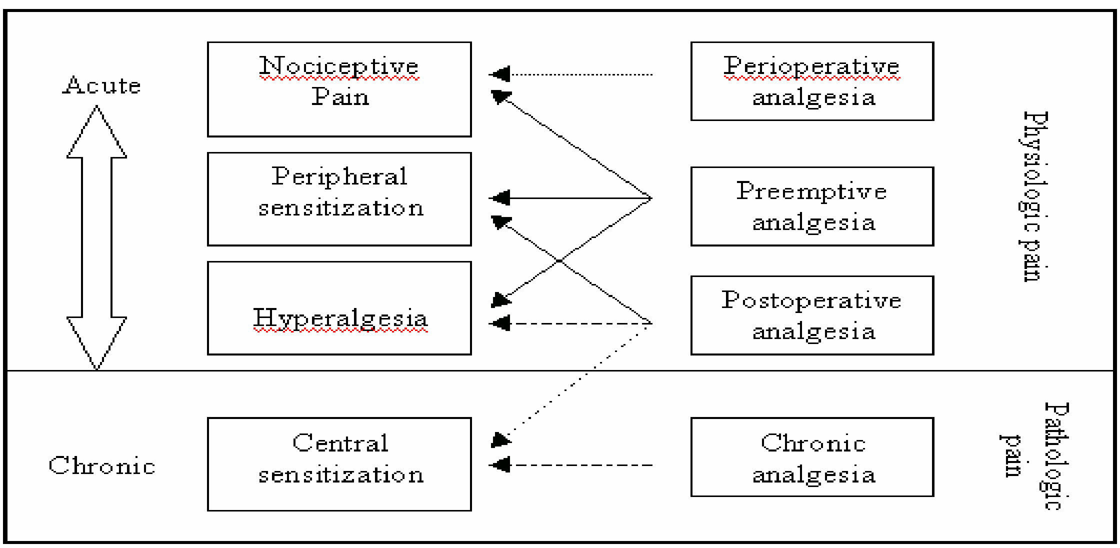

Fig 5.2.3: Analgesic strategies for pain management. The temporal progression of pain and approaches (and their targets) to the initiation and maintenance of analgesia. Arrows show effector pathways (dotted lines indicate lower efficacy). Adapted from Kissin I, Preemptive analgesia, *Anesthesiology* 2000; 93: 1138–1143.

The following is a guide to the species variability of animal in response to pain. It is important to note that individual variation also exists and may be especially pronounced in higher forms of animals like the nonhuman primates.

- Nonhuman primates — hunched posture, failure to groom, refusal of food or water, dejected appearance.
- Mice — withdrawal, biting response, piloerection, hunched posture, sunken eyes and abdomen, dehydration, weight loss.
- Rats — vocalization, struggling, licking/guarding, weight loss, piloerection, hunched posture, hypothermia.

- Rabbits — reduced eating and drinking, facing towards back of cage, limited movement, apparent photosensitivity.
- Pigs — vocalization and/or the lack of normal social behaviour, reluctance to move.
- Sheep and goats — rigid posture and reluctance to move.

Analgesic agents (Table 5.2.1) include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and narcotics. NSAIDs belong to a group of drugs having analgesic, antipyretic and anti-imflammatory activity due to their ability to inhibit the synthesis of prostaglandins. It includes aspirin, paracetamol, phenylbutazone, carprofen and ibuprofen. Meanwhile, glucocorticoids such as dexamethasone are also used for inflammation but care should be taken especially when the animal is pregnant as these can terminate the pregnancy in some species. Narcotics such as opiod derivatives morphine and etorphine are more potent than NSAIDs and glucocorticoids, but they can cause sedation, reduced GI motility, respiratory depression and they lack anti-inflammatory activity. Fentanyl transdermal patch can also be used for chronic management of pain in certain animals like dogs, pigs and rabbits. They are not generally used in nonhuman primates (NHPs) due to difficulty in preventing removal by the dexterous hands of the animals. Use in sheep is also problematic since the natural lanolin secretion from their skin reduces the adherence of the patch, reducing the potential for transdermal absorption.

Table 5.2.1: List of drugs commonly used in the laboratory animal

Drug	Mice	Rats	Rabbit	Pigs	Sheep	Nonhuman primate	Hamsters
Buprenorphine	0.05-0.1 mg/kg SC, IV q8-12h	0.1-0.5 mg/kg SC, IV q12h	0.02-0.05 mg.kg SC, IM, IV q8-12h	0.05-0.1 mg.kg IM, SC q8-12h	0.005 mg/kg SC, IM q8-12h	0.01-0.03 mg/kg IM, SC q8-12h	0.05-0.1 mg/kg, SC, IM q8-12 h
Butorphanol	1-5 mg/kg SC q6-8h	0.05-2.0 mg/kg SC q4h	0.1-0.5 mg/kg IV q4h	0.1-0.3 mg/kg SC, IM, IV q6-8h	0.3 mg/kg SC, IM, IV q12h	0.025 mg/kg IM q3-6h	1-5 mg/kg SC, Im q2-4h
Flunixin meglumine	2.5 mg/kg SC, IM try q12h	1.1 mg/kg SC, IM q12h	1.1 mg/kg SC, IM try q12h		0.5-1.0 mg/kg IV, IM q8h	0.5 mg/kg IM q24h	2-5 mg/kg, SC, IM q12-24h
Morphine	2.5 mg/kg SC q6-8h	10 mg/kg SC q2-4h	2-5 mg/kg SC IM q2-4h	0.2-0.9 mg/kg SC	-------	1-2 mg/kg IM, SC q4h	2-5 mg/kg, SC, IM q2-4h
Acetaminophen	300 mg/kg PO	110-300 mg/kg PO	1ml elixir in 100ml drinking water	-------	NR	10 mg/kg PO q8h	-------
Asprin	20 mg/kg SC, 100-120 mg/kg PO	20 mg/kg SC, 100-120 mg/kg PO	100 mg/kg PO q12h	10-20 mg/kg PO q8h	-------	10-20 mg/kg PO q6h	100-150 mg/kg PO q4h
Carprofen	5 mg/kg SC q24h	5 mg/kg SC q12-24h	1.5 mg/kg PO q12h	0.5-4.0 mg/kg SC q24h	4 mg/kg SC q72h	2 mg/kg PO SC q12-24h	5 mg/kg, SC, PO q12h
Ketoprofen	5 mg/kg PO, SC q24h	5 mg/kg PO, SC q24h	1 mg/kg IM	1.0-3.0 mg/kg SC, IM q24h	3 mg/kg IV, IM q24h	2 mg/kg IV IM q24h	2 mg/kg, SC q2-4h

Abbreviations: SC – subcutaneously, IV – intravenously, IM – intramuscularly, PO – orally, NR – not recommended.

Aside from management of pain using chemicals, cold compresses can also be intermittently applied on the region of interest in the first 24 to 48 hours. Application should just be long enough to produce vasoconstriction and can be as short as 20 seconds. Environmental considerations like placing cushions on the flooring to alleviate the pressure and prevent pressure sores and adding enrichment devices can also be done.

CHAPTER 5.3

ANIMAL FEEDS AND NUTRITIONAL REQUIREMENTS

Peik Khin Tan

Adequate nutrition for laboratory animals consists of water and food containing nutrients essential to provide energy and raw materials for growth, maintenance and repair of body tissue. Food items are composed of water, proteins, fats, carbohydrates, vitamins and minerals. Each nutrient type plays specific roles for different body processes are essential to animal health and well-being.

Nutritional requirements vary depending on species (e.g., herbivores, carnivores, omnivores), stages of life (e.g., reproduction, growth, maintenance), health status or condition (e.g., allergies, urinary tract infection), research protocol (e.g., *ad libitum*, restricted amount), gender and environmental condition (e.g., temperature, humidity).

Most laboratory animal diets contain 18 to 25 % crude protein of animal or plant origin, providing all essential amino acids in the right proportions. The largest percentage of total diet is usually carbohydrates, usually vegetable origins, mainly cereals, which serves as a source of energy. Most diets are composed of 2 to 8 % fat from plant or animal origin, which serves as a vehicle for fat-soluble vitamins and provides essential fatty acids. Fiber contents in all diets are made from natural ingredients. Water-soluble vitamins are usually found in the non-fatty tissues of plants and animals while fat-soluble vitamins can be obtained from the fatty parts and oils in plants. Minerals such as calcium and phosphorus are required for teeth and bone growth. Sodium and potassium play an important role in acid base balance. Any deficiency or excess in vitamins and minerals can cause serious disease.

Animals are provided with clean feed and water free of pathogenic organisms and harmful chemicals. The commercially available animals' feeds must meet the nutritional requirements of each species. Feed and water supplied to animals are tested quarterly to ensure they are safe and the nutrients values are within the ranges stated on the label of the feed bag.

Rodents, rabbits, pigs, sheep and geese are fed pelleted diets while tree shrew and monkeys are fed monkey chow formed into biscuits. Specific Pathogen Free (SPF) rodents are given autoclaved food and water. Caution must be taken though when using autoclaved feeds as the moisture during the autoclaving process can facilitate fungus build-up, vitamins can be destroyed and proteins can carmalize, making the pellets hard. Irradiated feeds (gamma radiation) are also available commercially.

The daily feed and water requirements for laboratory animals are listed in Table 5.3.1.

Table 5.3.1: Daily feed and water requirements for selected laboratory animals

Species	**Daily feed intake (gms)/100 g BW/day**	**Daily water intake (ml)/100 g BW/day**
Mouse	12–15	15
Lactating Mouse	80–100	80–100
Hamster	5	10
Rat	5	10
Rabbit	5	10
Lactating Rabbit	10–15	Up to 90
Monkey (g/kg BW/d)	350–550	350–950
Domestic Swine (kg/d)	3.6–4.1	80–120
Sheep (g/kg/d)	15–60 (dry matter intake)	197

Nutritional Requirements for Rodents

Rodents (mice and rats), being omnivorous, are fed with 12 mm diameter rat and mouse pellets fortified with vitamins and minerals to meet the requirements of these animals after the diet is autoclaved (Fig 5.3.1). The diet is made of wheat, lupins, barley, soya meal, fish meal, mixed vegetable oils, canola oil, salt, calcium carbonate, dicalcium phosphate, magnesium oxide, vitamin and trace mineral premix to contain 19.6 % protein, 4.6 % total fat, 4.5 % crude fiber and 14.3 mj/kg digestible energy. This commercially available rat feed is also generally used as he basic diet for hamsters, sometimes in combination with rabbit food to provide a balance of 16 to 24 % protein, 60 to 65 % carbohydrate and 5 to 7 % fat.

Fig 5.3.1: Rodent pellet.

The added vitamins and trace minerals are as follows (Table 5.3.2):

Table 5.3.2: List of added vitamins and minerals in mouse and rat diet

Added vitamins and minerals in the mouse and rat diet	
Vitamin A (Retinol)	10,000 IU/Kg
Vitamin D3 (Cholecalciferol)	2000 IU/Kg
Vitamin K (Menadione)	20 mg/kg
Vitamin E (α Tocopherol acetate)	100 mg/kg
Vitamin B1 (Thiamine)	80 mg/kg
Vitamin B2	30 mg/kg
Niacin (Nicotinic acid)	100 mg/kg
Vitamin B6 (Pyridoxine)	25 mg/kg
Calcium Pantothenate	50 mg/kg
Biotin	300 mg/kg
Folic acid	5 mg/kg
Vitamin B12 (Cyanocobalamin)	150 μg/Kg
Magnesium	100 mg/kg
Iron	70 mg/kg
Copper	16 mg/kg
Iodine	0.5 mg/kg
Manganese	70 mg/kg
Zinc	60 mg/kg
Molybdenum	0.5 mg/kg
Selenium	0.1 mg/kg

Nutritional Requirements for Rabbits

Rabbits are herbivorous and given 4-mm diameter, 4 to 10 mm long meat-free guinea pig and rabbit pellets and timothy hay twice a day. The diet (Fig 5.3.2) is fortified with vitamins and minerals to meet the daily nutritional requirement. The diet is composed of lupins, oaten hay, Lucerne, barley, soya meal, canola meat, DL Methionine, mixed vegetable oils, salt, dicalcium phosphate, magnesium oxide, a vitamin and trace mineral premix to make up 18.2 % protein, 4.2 % total fat, 14.4 % crude, 18 % acid detergent fibers and 11 mj/kg digestible energy.

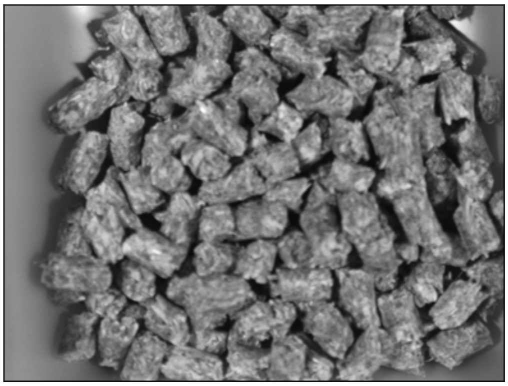

Fig 5.3.2: Rabbit feed.

The added amino acids, vitamins and trace minerals are as listed in Table 5.3.3:

Table 5.3.3: List of added vitamins and minerals in rabbit diet

Added vitamins and minerals in the rabbit diet	
Vitamin A (Retinol)	49, 000 IU/kg
Vitamin K (Menadione)	3 mg/kg
Vitamin E (α Tocopherol acetate)	60 mg/kg
Vitamin B1 (Thiamine)	5.6 mg/kg
Vitamin B2	6.6 mg/kg
Niacin (Nicotinic cid)	56 mg/kg
Vitamin B6 (Pyridoxine)	5.8 mg/kg
Pantothenic acid	19 mg/kg
Biotin	140 μg/kg
Folic acid	0.6 mg/kg
Vitamin B12 (Cyanocobalamin)	7 mg/kg
Choline	2,200 mg/kg
Iron	40 mg/kg
Copper	13 mg/kg
Iodine	1.7 mg/kg
Manganese	90 mg/kg
Cobalt	0.7 mg/kg
Zinc	60 mg/kg
Selenium	0.1 mg/kg

Nutritional Requirements for Nonhuman Primates (NHPs)

All nonhuman primates (NHPs) require Vitamin C in their diet like man and guinea pigs. These species of animals cannot synthesize vitamin C unlike most because they lack the enzymes necessary for conversion of L-gluconolactone to L-ascorbic acid and cannot store the vitamin in any appreciable extent. Lack of Vitamin C leads to scurvy, which causes formation of livid spots on the skin, spongy gums, and bleeding from almost all mucous membranes.

Nutritional requirements vary from Old World Monkey to New World monkey. *Macaca fascicularis* (cynomolgus or crab-eating macaque) is under Old World Monkey species and fed with Laboratory Fiber-Plus® Monkey Diet (Fig 5.3.3). The diet is made of ground corn, dehulled soybean meal, ground soybean hulls, ground oats, corn gluten meal, ground wheat, animal fat preserved with BHA, dehydrated alfalfa meal, sucrose, dicalcium phosphate, dried whey, fish meal, calcium carbonate, brewers dried yeast, salt, L-ascorbyl-2-polyphosphate, pyridoxine hydrochloride, menadione dimethylpyrimidinol bisulfite, cholecalciferol, DL-methionine, choline chloride, vitamin A acetate, folic acid, calcium pantothenate, ferrous sulfate, dl-alpha tocopheryl acetate, biotin, thiamin mononitrate, nicotinic acid, riboflavin, cyanocobalamin, zinc oxide, L-lysine, manganese

Fig 5.3.3: Monkey Chow.

oxide, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate, sodium selenite. This diet contains 20 % crude protein, 5 % crude fat, 10 % crude fiber, 3 % added minerals and 5.5 % ash.

Monkey Chow must be used within 180 days of manufacture, assuming it contains a stabilized form of Vitamin C, otherwise it must be used in 90 days. The stability of Vitamin C varies with environmental conditions, therefore special care must be taken to store feed properly. Monkeys generally consumes about 2 to 4 % of their body weight in food each day. The daily food allowance is given in equal portions twice during the day to prevent wastage. Fresh, clean water is available at all times from an automatic watering system. Laboratory Fiber-Plus® Monkey Diet is sometimes soaked in fruit juice to soften the product for infants or animals that have difficulty chewing.

Fruits such as bananas, apples, grapes and oranges are given once a day for primate enrichment, assuming it does not interfere with the research protocol.

Nutritional Requirements for Swines

Gold Coin Company from Malaysia supplies swine feed (Fig 5.3.4). The feed is made of yellow maize, molasses sugarcane, canola meal, high protein soyabean meal, palm oil, rice bran, wheat pollard, L-lysine, methionine, L-threonine, dicalcium phosphate, limestone dust, salt, sodium bicarbonate pig mineral mix, choline chloride liquid, and pig vitamin basemix. The diet contains 17.5 to 19.5 crude protein, 3.5 to 7 % ether extract, 2.5 to 5 % crude fiber, 0.9 to 1.1 % calcium, 0.6 to 0.8 % phosphorus and 13 % maximum moisture content. The diet is fortified with minimum values of 7 IU vitamin A, 1.2 IU vitamin D, 10 g vitamin E, 1 g vitamin K, 100 g zinc, 150 g copper and 100 g ferrous per ton of feed. Swine consume 1 to 4 % of body weight. Swine do not require elemental sulfur since they utilize sulfur-containing amino acids. The water is supplied by automatic watering system *ad libitum*.

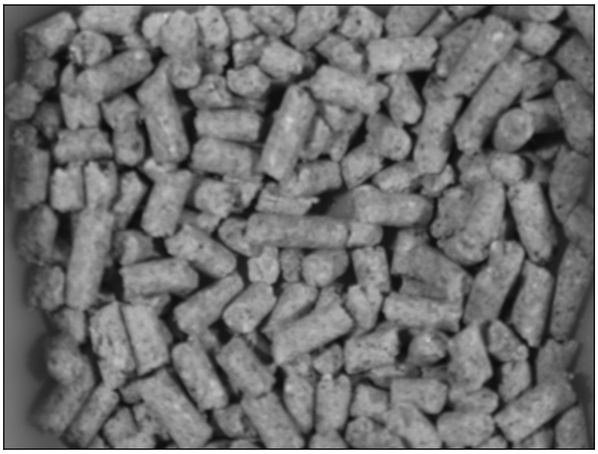

Fig 5.3.4: Pig pellet.

Nutritional Requirements for Other Laboratory Animals

The nutritional requirements of tree shrews are not well documented at this moment although they are fed once daily with LabDiet® monkey chow. Fruit supplements like bananas, apples and pears are provided as well. Each tree shrew is to be provided with 14 g of food (2 monkey chows) per day. Commercial dog food is also found to be suitable for these animals.

Geese are fed once daily with Gold Coin Pig Grower Feeds unless otherwise instructed. Feeds like grass are provided as often as possible. On zero grazing, geese will eat up to 200 g of food per day (depending on the size of the goose).

Sheep feed is also supplied by Gold Coin and it contains minimum crude protein 15 to 17 %, maximum crude fiber 12 %, minimum crude fat 3 %, maximum moisture 13 %, maximum ash 12 %, calcium 0.8 to 1.4 %, and phosphorus 0.5 to 0.9 %. The sheep feed is made of molasses, wheat, soyabean meal, palm oil, rice bran, cocoa cake, wheat pollard, sodium bicarbonate mineral, choline chloride, and vitamin basemix. Sheep are also given high quality hay from Australia. The hay provides 14.2 % protein, 7.4 % ash and minerals.

It is very important to note that sheep is unique among food and farm animals in the way they utilize copper. Copper, a required mineral, is potentially toxic to all food animals although sheep is most susceptible. Its metabolism is affected by the presence of other minerals and some ionophores, especially the levels of molybdenum and sulfur, which act as its antagonists. These compounds bind with copper and prevent gut absorption and increase excretion of absorbed copper in the liver and body tissues. Prevention of copper toxicity involved not feeding sheep any swine, cattle or poultry rations, which contain high levels of copper by design.

The feed samples are analysed once a year to ensure the nutrient composition mentioned by manufacturers contains in the feed.