Guideline 20

Guidelines for the Housing of Rats in Scientific Institutions

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Recommendations

The following recommendations appear in the body of the text:

1.2.1 Institutions using rats for scientific purposes are responsible for responding effectively to recommendations of the institution's Animal Ethics Committee to ensure that facilities for the housing and care of rats are appropriate to the maintenance of well-being and health of the rats.

1.3.1 The chief investigator/teacher (person in charge of a research/teaching project) has personal responsibility for all matters related to the welfare of rats under his or her control, which includes their housing and care. (As per the principle contained in Clause 3.1.1 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes)

1.3.2 The chief investigator/teacher should ensure that the extent of personnel / staff supervision is compatible with the level of competence of each person and the responsibilities they are given in relation to rat care and management. (As per the principle contained in Clause 3.1.2 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes)

1.5.1 To meet the requirements of the Code of Practice (ie to provide accommodation that meets the species-specific needs of rats), housing should be provided which allows rats the opportunity for social interaction, the opportunity to carry out normal behaviours and the opportunity to rest and withdraw from each other.

1.5.2 The Code of Practice recognises that there may be circumstances where the requirements of experimental procedures will preclude meeting some species-specific needs (Clause 4.4.19). Housing in these situations should still meet the physiological and psychological needs of rats as closely as possible.

2.2.1 As a guide, based on the information from Scharmann 1991, Patterson-Kane 2002 and Koolhaas 1999, the minimum floor area for a group of up to 5 rats of up to 250-300gm body weight should be 1,500cm^2 and preferably 1,800cm^2. For larger rats, group size should be decreased or cage floor area increased, on the basis that as rats grow, while play behaviour decreases, cage floor area must accommodate other behaviours including social interaction.

2.2.2 As a guide, for a nursing mother and litter (up to weaning at about 21 days), the floor area should be a minimum of 1,500cm^2.

2.2.3 As a guide, for juvenile rats (from weaning to about 50 days), for a maximum group of 12 juveniles, the floor area should be a minimum of 2,000cm^2.

2.3.1 Ideally the height of cages should allow rats to stand on their hind legs and stretch up fully. This height does not need to be provided over the entire area of the cage.

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2.3.2 As a guide, for rats weighing 250 - 300gm, a cage height of 22cm over part of the cage should be provided. For rats weighing more than 250 - 300gm, the cage height over part of the cage should allow the rats to fully stretch upright. However, it is recognised that currently available cages (with maximum heights of around 22cm - 24cm) are unlikely to accommodate this.

2.3.3 Where cages are fitted with platforms or in-cage shelters, the distance between the top of the platform or in-cage shelter and the top of the cage should be at least 8cm to allow rats to climb onto the top of the platform or in-cage shelter.

2.3.4 While cage height (over part of the cage) should allow for upright standing behaviour, food and water should be accessible at a level that allows rats (especially juvenile rats) to sit while eating and drinking, to avoid bony and cartilaginous damage.

2.4.1 Rectangular rather than square cages should be provided.

2.5.1 Rat cages should be made from plastic (for example polypropylene, polycarbonate, polysulphone, polyetherimide) floors and walls ("shoebox" or "tub") with wire mesh tops (unless special purpose cages such as filter top cages or individually ventilated cages are required).

2.5.2 Where transparent plastics are used for cage "tubs", particular attention should be paid to providing rats with shelters to allow them to withdraw from light and activities outside their cage.

2.5.3 The need to monitor rats by observation (the intensity of which will be dictated by the type of project), and the disturbances to rats that may occur in doing this in opaque cages with opaque shelters, needs to be balanced against preferences for opaque walls by rats.

2.5.4 Rat cages should be fitted with "high top" wire mesh lids (on solid sided walls) which enable rats to stretch upright and which facilitate interaction by rats with their surrounding environment (via visual and olfactory inputs). The "high top" area does not need to extend over the entire roof of the cage.

2.6.1 Solid floors should be provided for rat caging.

2.6.2 Wire mesh floors should not be used for rat caging unless with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to use such flooring. In such cases, a solid floor section and nesting material should preferably be provided. The size of the mesh gaps should not exceed 11mm x 11mm. (See also 3.3 Metabolism Cages)

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2.7.1 Bedding should be provided in rat cages and should be in sufficient quantity to cover the whole floor. The depth of bedding required will vary with factors such as the type of bedding used, the number of rats in the cage and the frequency of cleaning. As a guide, the depth of bedding should be at least a minimum of 2 cm.

2.7.2 Ideally bedding should be free of dust, microbial, parasitic, or chemical contaminants, non-traumatic, moisture absorbent and ammonia binding. The properties of bedding provided should also include that its particles can be manipulated and/or that it is suitable for digging / burrowing.

2.7.3 In choosing bedding, the potential for bedding types to induce hepatic enzymes needs to be taken into consideration.

2.8.1 All rats should be provided with nesting material in addition to bedding material.

2.8.2 Nesting material should be loose, manipulable and light enough to be carried. Suitable materials include shredded paper, straw and woodwool.

2.8.3 The way in which nesting materials are provided (eg top of cage lid versus within the cage) should take into account stain-specific differences in the use of materials, depending on the site where they are provided.

2.9.1 Rats should be provided with a shelter within their cage.

2.9.2 In-cage shelters should ideally have solid, opaque sides and roof that allow withdrawal from the light (and from other rats) and should be constructed so that rats are able to climb onto the roof of the shelter.

2.9.3 Where in-cage shelters are made of chewable material, it should be ensured that the material is not toxic to rats.

2.9.4 The minimum space between the roof of the shelter and the top of the cage should be 8cm to allow for rats climbing onto the roof of the shelter.

2.10.1 The use of pens and enlarged cages, with furnishings such as shelters, ledges and ramps, may be considered as a viable alternative to conventional caging for rats.

3.1.1 As a guide, optimal numbers for groups of adult rats is probably up to 4 individuals. In deciding optimal group sizes, factors such as differences between individuals, strain, sex and cage size should be taken into account.

3.1.2 Juvenile stock rats may be housed in groups of up to 12 rats (preferably as litter mates) until approaching sexual maturity (around 50 days of age) (Lawlor 1987).

3.1.3 Ideally rat groups should be made up of litter mates of the same sex.

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3.1.4 Rats should be grouped with each other before they reach puberty to avoid or minimise problems of aggression between unfamiliar individuals (Hurst, Barnard, Nevison et al 1999).

3.1.5 The disruption of established social groups should be avoided.

3.1.6 When removing rats from, and reintroducing them to, cage mates, it should be aimed to keep the period of separation to 48 hours or less (to take advantage of social memory).

3.1.7 Where possible, adult males from different groups should not be placed in the same cage.

3.1.8 Where it is necessary to mix unfamiliar adult males, they should be exposed to each other before they are mixed together. This can be achieved by placing the newcomer into an adjoining cage with visual and olfactory contact with the other male.

3.1.9 Shelters should be provided within cages to enable rats to hide in case of conflict.

3.1.10 Groups of rats should be monitored to ensure social stability as well as the detection of behavioural and physiological abnormalities. In monitoring rats for "real fighting" versus "play fighting" indicators during skirmishing such as the target of contact (rump versus the nape of the neck), hard biting and raised hairs (piloerection) can be used (as well as measurement of the frequency of ultrasonic vocalisations).

3.2.1 Rats should not be housed individually unless with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to house rats in this way. In such cases, rats should be able to be in visual, auditory and olfactory contact with other rats.

3.3.1 Rats should not be housed in metabolism cages unless with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to house rats in this way. In such cases, rats should be able to be in visual, auditory and olfactory contact with other rats as far as possible.

3.3.2 Rats should be acclimatised to the metabolism cage before studies commence.

3.3.3 Where metabolism cages have to be used, consideration should be given to enriching the cages (for example with an area of solid floor and a nest box), providing this does not interfere with the study.

3.4.1 Principles and recommendations related to housing rats in conventional cages apply to housing rats in individually ventilated cages.

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3.5.1 Steps should be taken to allow rats to become familiar with the people who will be handling them so as to reduce the stress of handling. This should include the process of "gentling" (whereby rats are allowed to explore their handler and are gently stroked and held).

3.5.2 "Gentling" (habituation to handling) of rats should not be linked with (ie neither proceed nor follow) procedures that may cause distress to rats.

3.5.3 Handling rats for routine husbandry should not be linked with (ie neither proceed nor follow) procedures that may cause distress to rats.

3.5.4 To reduce the effect of stress responses on rats and subsequently the effects on data collection, rats should be habituated to their surroundings and to routine procedures.

3.5.5 Handling rats at all times should be done quietly and gently.

3.5.6 Experimental procedures should be scheduled taking into account the potential effects on rats of routine husbandry procedures.

3.5.7 The training and rewarding of rats using positive reinforcement or "treats" should be considered when performing procedures on rats. This is likely to reduce the stress on rats and increase their co-operation.

3.6.1 Rats should be provided with items to enrich their environment. Items that assist rats to perform each of the five following categories of behaviours should be provided:

- *social interaction (see Section 3.1 The Social Environment),*
- *chewing/gnawing,*
- *locomotion (including climbing, exploring and playing),*
- *resting/hiding, and*
- *manipulating, carrying and hoarding food and objects.*

(See 3.5 (xiv))

3.6.2 When techniques are used in an effort to provide environmental enrichment for rats it is important that the success of the techniques, in terms of improving the rats' welfare, is evaluated.

3.7.1 Where it is necessary to individually identify rats, the least invasive method that is compatible with the use of rats should be used.

3.7.2 Non-toxic dyes and permanent markers may be used on the fur and tail. These methods of identification usually need to be replaced every two weeks.

3.7.3 Fur clipping may be used but needs to be carried out frequently.

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3.7.4 Subcutaneous microchipping, tattooing and ear notching may be used where permanent identification is necessary. Note there is some transitory pain associated with applying these forms of identification and the use of anaesthesia and/ or analgesia should be considered..

3.7.5 Toe and tail tip amputation are painful procedures and should not be used.

3.8.1 A nutritionally adequate diet should be provided for rats.

3.8.2 Food and water should be provided ad libitum unless special permission has been obtained to vary this regime from the Animal Ethics Committee of the institution.

3.8.3 Variations in the types of food and how it is presented should be provided (for example, commercial pellets, dried sunflower seeds, corn on the cob, fresh vegetables).

3.8.4 Food items should be provided not only in food hoppers but should also be sprinkled onto the cage floor bedding to add interest, foster foraging behaviour and promote normal postures during feeding.

3.8.5 The rat's nocturnal feeding patterns should be taken into account in study design, especially when treatments are given in the diet.

3.9.1 Welfare monitoring of rats via behavioural observation should be carried out in addition to monitoring for general physical health.

3.9.2 Monitoring should be carried out when a person with whom the rats are familiar is present.

3.9.3 In the monitoring and investigation of heath issues (such as growth rate, reproductive performance and disease) the effects of housing conditions should be taken into account.

4.2.1.1 Lighting within cages during day hours should be held at lux ranges below thresholds of aversion for rats. For most pigmented rat strains this is below 60 lux and for albino rats below 25 lux. To enable operators in rat rooms to perform visual tasks, it may be necessary to increase light levels (to approximately 210 lux at working height) for the period that the operators are in the rooms.

4.2.1.2 Light intensity can be reduced by using recessed lighting consoles in the ceiling with fluorescent lights of about 25 to 36 Watt and a low spectral intensity (wavelength) (which can be achieved by using a low colour number (for example colour 33 tubes)).

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4.2.1.3 Shading should be provided over the top shelves of racks to protect rats in the top cages from overhead lights and to provide a more uniform light level between cages on different shelves.

4.2.1.4 Lighting should be diffuse and uniform to avoid glare, heat clusters and fluctuating lighting conditions for individual cages

4.2.1.5 Under bright operating lights the eyes of rats of any strain should be protected to prevent retinal damage.

4.2.1.6 Lights should be checked for flickering and any flickering rectified.

4.2.2.1 Regular light cycles of 12/12 – 10/14 hours light/dark are suggested. Variations in the light dark cycles to mimic seasonal changes could be considered.

4.2.2.2 The use of dimmers in rat rooms is suggested to allow the creation of twilight periods between the light and dark cycles.

4.3.1 A room temperature range for rat housing between 20 - 26^{0}C is recommended.

4.3.2 Significant swings in room temperature should be avoided.

4.3.3 Rats should be provided with nesting materials and in-cage shelters to enable them to regulate the microclimate temperature, particularly for sleeping

4.3.4 Special attention should be given to those circumstances where the rat's thermoregulatory ability is compromised. Cage temperature for pregnant and lactating rats and pups up to 3-4 weeks of age should be at the higher end of the recommended range (24-26^{0}C).

4.3.5 If rats are held in wire bottomed cages without some solid resting area and nesting material, (for example in metabolism cages) the room temperature should be in the range of 24-26^{0}C.

4.3.6 Temperature should be monitored within the cage and at various positions within the room to monitor variation so as to optimally manage the microenvironment.

4.4.1 A relative humidity at the level of rat cages of 40-70% is recommended.

4.5.1 The number of air changes per hour needs to be adjusted to keep air quality, temperature and humidity at acceptable levels within cages.

4.5.2 Room ventilation rates of about 15-20 air changes per hour may be needed.

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4.5.3 For rooms holding individually ventilated cages, usually 5 air changes per hour will be sufficient to maintain room air quality.

4.5.4 For individually ventilated cages, to ensure low levels of ammonia, air changes should be kept at around 50 times per hour (Krohn, Hansen and Dragsted 2003).

4.5.5 Racks should be positioned in a room so as to optimise air exchange and avoid animals being exposed to draughts.

4.5.6 Cleaning regimes should be managed to maintain ammonia levels within a cage below 25 ppm.

4.6.1 Noise (loud sounds) within the human hearing range as well as in the ultrasonic range should be reduced where possible.

4.6.2 Computers, or any other equipment likely to emit high frequency ultrasonic signals, should not be used in rooms where rats are housed. If the use of such equipment is unavoidable then measures, such as packing the equipment in polystyrene foam plating, should be taken to dampen ultrasonic noises.

4.6.3 The effect of background radio sounds to alleviate the effects of ultrasound and loud noises is unclear. If a radio is used, the volume should be kept low.

4.6.4 Vibrations in rat holding rooms, and especially of cages, should be eliminated.

4.6.5 Individually ventilated cages should be checked for vibrations.

4.6.6 Due to the vibrations created, placing motorised equipment on bench tops with cages should be avoided.

4.7.1 Rat rooms should have temperature and humidity read-outs in a position where staff can easily see them.

4.7.2 Sensors should be fitted to monitor and report malfunctions in ventilation, temperature and humidity control on a 24 hour basis, with automatic alarm activation.

4.7.3 Even if centralised computer systems are used to regulate the general environmental conditions, it is still essential to check these variables regularly at the cage level.

4.8.1 The need for changing bedding depends on the kind of bedding used and air quality. The frequency of bedding changes also will be influenced by stocking rates, strains of rats and particular disease conditions, for example, diabetes. As a guide, bedding is commonly replaced about once a week.

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4.8.2 Cleaning regimes should be managed to maintain ammonia levels within a cage below 25 ppm.

4.8.3 Cleaning of cages should be done in a separate room designated for maintenance and cleaning tasks. The cage washing area should not be located near rat holding rooms to minimise disturbance from the associated activities.

4.8.4 Rat rooms should have smooth, hard and impervious surfaces throughout with no exposed joints or cracks.

4.8.5 All surfaces should be washed down periodically to keep them clean.

4.8.6 Rat holding rooms should not contain floor drains and if they do they should be rodent proof.

4.8.7 Procedures to reduce the risk of disease spread during cleaning should be developed with particular attention to staff working in contaminated areas and with diseased animals.

4.8.8 Clean storage space for cages, food and bedding should be provided.

5.1.1 All cages should have labels attached to them that provide the following information, or cross reference to a central record in the same room containing this information:

- *Rat identification (strain, sex, number of rats)*
- *Age (date of birth) of litters or of individual rats.*
- *Date of entry into cage.*
- *Name and approval number of project in which rats are being used.*
- *Name, location and contact numbers of the chief investigator/teacher and, if applicable, other investigators/teachers using the rats.*
- *Name, location and contact numbers of staff associated with the housing and care of the rats.*
- *Treatments / procedures*

5.2.1 To assist in monitoring the management of rat breeding colonies, regular reports must be provided to the Animal Ethics Committee, for review, on the fertility, fecundity, morbidity and mortality of all rat breeding colonies. The frequency of such reports should be at least 6 monthly and more often if deemed necessary by the AEC. (See ARRP Guideline 16: Supervision of Animal Supply by Animal Ethics Committees - http://www.animalethics.org.au)

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1. General

1.1 Introduction

(i) These guidelines are intended for use by people involved in the housing and care of rats in scientific institutions. The guidelines are not intended to be a complete manual on rat care and management but rather to provide some key guiding principles on good contemporary practice in rat housing. The guidelines will be revised from time to time to take account of advances in the understanding of rat physiology and behaviour, technological advances, and changes in community attitudes and expectations about the welfare of animals.

(ii) The guidelines are based on principles regarding the care and management of rats taken from scientific literature. These principles are detailed throughout the document, as are recommendations for the care and management of rats which are derived from these principles. In some areas, conclusions to be drawn from the available literature are not entirely clear, and in such areas recommendations are extrapolated from information available and practices in rat care and management current at the time of writing.

(iii) The principles outlined in the document address requirements of the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (as outlined below in Section 1.4). The requirements of the Code of Practice include that animals held for scientific purposes should have their species-specific behavioural and physical needs met, whilst at the same time ensuring that the animals can adequately be monitored and are protected from disease, and taking into account the requirements of the research for which the animals are being used.

(iv) Whilst the guidelines focus on the welfare of rats, it is implicit that conditions that contribute to meeting rats' physiological and behavioural needs will also contribute to the quality of scientific outcomes. The guidelines contain many examples of the physiological and behavioural responses of rats associated with variables in housing and hence the potentially confounding effects of these variables on these animals as research subjects.

(v) The guidelines outline requirements for the housing of normal rats. Where rats are physically or behaviourally abnormal (for example, post-surgery, diabetics, Parkinson models, acute pain models), modifications to housing to meet their needs may be required.

1.2 Responsibilities of Institutions

Recommendations

1.2.1 Institutions using rats for scientific purposes are responsible for responding effectively to recommendations of the institution's Animal Ethics Committee to ensure that facilities for the housing and care of rats are appropriate to the maintenance of well-being and health of the rats.

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1.3 Responsibilities of Chief Investigators / Teachers

Recommendations

1.3.1 The chief investigator/teacher (person in charge of a research/teaching project) has personal responsibility for all matters related to the welfare of rats under his or her control, which includes their housing and care. (As per the principle contained in Clause 3.1.1 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes)

1.3.2 The chief investigator/teacher should ensure that the extent of personnel / staff supervision is compatible with the level of competence of each person and the responsibilities they are given in relation to rat care and management. (As per the principle contained in Clause 3.1.2 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes)

1.4 The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes

Principles

(i) The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states:

4.4.19 Animal accommodation should be designed and managed to meet species-specific needs. Pens, cages and containers should ensure animal wellbeing and comfort. Variations to these requirements as part of a project must receive prior AEC approval. The following factors should be taken into account:

(i) species-specific behavioural requirements, including the availability and design of space to enable free movement and activity, sleeping, privacy, contact with others of the same species, and environmental enrichment;

(ii) provision of single housing for animals when appropriate for the species and if necessary for the purpose of the project (for example, during recovery from surgery or collection of samples);

(iii) species-specific environmental requirements, such as lighting, temperature, air quality, appropriate day/night cycles and protection from excessive noise and vibrations;

(iv) the need to provide ready access to food and water;

(v) the need to clean the pen, cage or container;

(vi) protection from spread of pests and disease;

(vii) requirements of the project; and

(viii) the need to observe the animals readily.

4.4.20 Pens, cages and containers must:

(i) be constructed of safe, durable, materials;

(ii) be kept clean;

(iii) be maintained in good repair;

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(iv) be secure and escape-proof;

(v) protect animals from climatic extremes;

(vi) not cause injury to animals;

(vii) be large enough for the species and the number of animals held; and

(viii) be compatible with the behavioural needs of the species.

4.4.21 The number of animals in cages, pens or containers and the placement of these should enable social and environmental conditions for the species to be maintained. Where it is necessary to individually house animals of a species that normally exists in social groups, the impact and time of social isolation should be kept to a minimum.

4.4.22 Bedding and litter must be provided if appropriate to the species and should be comfortable, absorbent, safe, non-toxic, able to be sterilised if needed, and suitable for the particular scientific or educational aims. Pregnant animals must be provided with nesting materials, where appropriate.

4.4.23 The AEC, investigators and teachers should be consulted in advance of planned changes to these conditions, since these may affect both the welfare of animals and results of the scientific and teaching activities.

1.5 Aspects of Rat Behaviour Relevant to Housing

Principles

(i) The most commonly used laboratory rats have evolved from the Norway Rat, *Rattus norvegicus* which lives mainly in burrow systems in the ground (Koolhas 1999). Both wild and domestic rats will create complex, three dimensional burrow environments (Brain 92; Boice 1977).

(ii) Rats are social animals. In the wild they live in colonies, which may consist of hundreds of rats and which have nesting sites and feeding grounds in common (Barnett 76).

(iii) Rats are nocturnal, usually with three activity periods, one at the beginning, one in the middle and one at the end of the night. Feeding for both adults and neonates takes place during these activity periods (Henning and Gisel 1980; Koolhas 1999).

(iv) Rats have highly developed senses of smell, hearing and touch. Rat behaviour and communication is strongly influenced by olfactory cues (Mackay-Sim and Laing 1979; Kikusui, Takigami, Takeuchi and Mori 2001; Koolhas 1999).

(v) Rats emit sonic as well as ultrasonic vocalisations and can hear frequencies at least up to 70kHz (Heffner et al 1994). The use of ultrasound appears to be important for communication and may be used in behaviours such as controlling aggressive encounters, mating and mothering (Sales and Pye 1974; Gamble 1982, Brudzynski and Ociepa 1992).

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(vi) Normal behaviours of rats include climbing, walking, standing on their hind legs and stretching upright, burrowing, nesting, gnawing, foraging, grooming (themselves and each other) and retreating (into hiding areas).

(vii) Young rats, and sometimes even older rats, engage in play behaviour which includes leaping, chasing and scuffling (Scharmann 1991). Play behaviour is necessary for the well-being and normal social and sexual development of young rats (Lawlor 2002). One function of play may be to establish stable social relationships (Panksepp 1981).

(viii) Rats under laboratory conditions have been observed to spend about 70 to 75 % of their time resting (Manser, Morris and Broom 1995).

(ix) Rats usually sleep in relatively curled positions (van Betteray, Vossen and Coenen 1991) but have been observed to sleep stretched out at full length with their tails extended (Lawlor 2002). Sleeping positions may be influenced by factors including light, temperature and proximity to walls.

(x) Huddling behaviour of rats (sleeping together in a group) is influenced by needs for thermoregulation but is not solely caused by this. Other sensory stimuli (for example olfactory and/or tactile) appear to play a part in huddling behaviour (Barnett 1976; Sokoloff and Blumberg 2001).

(xi) Rats are highly exploratory and inquisitive (Scharmann 1991; Barnett 1976). Studies have shown that rats will work for stimulation and that they demonstrate preferences for the opportunity to explore (over a blind alley) and for novel visual stimuli (Barnett 1976).

(xii) The normal behaviour of rats when eating is to carry a piece of food by their teeth to a suitable spot where they adopt a squatting posture and hold the food in their forepaws to nibble at it (Lawlor 2002).

(xiii) Gnawing is a behaviour that is necessary not only for the psychological (Beltz, Kennell, Czambel et al 2003) but also for the physiological well being of rats. If not given the opportunity regularly to gnaw, their teeth overgrow, which can make eating and grooming difficult or impossible.

(xiv) Rats exhibit coprophagy – they will ingest 35-65% of their faeces when fed a complete diet. They eat faeces directly from the anus and have been shown to experience growth depression if prevented from doing this (this effect seems to be related to the consumption of fresh faeces, as eating faecal pellets from the bottom of the cage has no beneficial effect on growth (Newton 1978)). Young rats ingest maternal faeces between 16 and 28 days of age. This significantly decreases after 25 days of age, coinciding with weaning. Young pups deprived of maternal faeces show evidence of malnutrition and abnormal eating behaviours when older (Novakova and Babicky 1989).

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(xv) For housing rats, in addition to the normal requirements of rodents for food, water, exercise, shelter and warmth, particular consideration should be given to:

* Their nocturnal habits
* Their ultrasonic hearing
* Their response to pheromones
* Their response to isolation
* Their exploratory nature
* Their upright-stretching bi-pedal posture
* The effect of group size on social dynamics
* The influence of strain, age, sex and prior experiences on behaviour.

(xvi) Assessments of rat behaviour need to take into account that the behavioural and physiological responses of rats will vary with factors including sex, age, strain, prior experiences and the environments in which they are kept (Galef and Sorge 2000; Brown and Grunberg 1995; Chaouloff, Kulikov, Sarrieau et al 1995; Rebouças and Schmidek 1997; Gomez, Kloet and Armario 1998; Dimitrijevic, Laban, Djuric et al 2001; Hall, Huang, Fong et al 2000; Fernandes, Gonzalez, Wilson and File 1999; Rose 1996).

Recommendations

1.5.1 To meet the requirements of the Code of Practice (ie to provide accommodation that meets the species-specific needs of rats), housing should be provided which allows rats the opportunity for social interaction, the opportunity to carry out normal behaviours and the opportunity to rest and withdraw from each other.

1.5.2 The Code of Practice recognises that there may be circumstances where the requirements of experimental procedures will preclude meeting some species-specific needs (Clause 4.4.19). Housing in these situations should still meet the physiological and psychological needs of rats as closely as possible.

2. Cage Design

2.1 Living Area

Principles

(i) The living area for rats is three dimensional, comprised of the floor area as well as the vertical space. These parameters need to be considered together, rather than in isolation. In addition to three dimensional space, the shape of the living area and the size of groups of rats (see 3.1 The Social Environment) need to be taken into account in developing optimal living areas.

(ii) The living area for rats must allow them to satisfy their basic physiological and behavioural needs and these include the ability to rest, groom, search for food, explore, gnaw, hide, reproduce and engage in a range of social activities including play (Brain 1995).

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(iii) Information on the requirements of rats for living space is not conclusive. Rats' requirements for height and shape of the living area are reasonably well documented. However, their requirements for floor area are less clear.

(iv) The parameters of cage size, space available per rat (spatial density) and number of rats in the group (social density) are often confounded in housing studies (Hargreaves 2000). There is some evidence to indicate that housing rats at low amounts of space per rat or high group numbers can influence physiological and behavioural systems and that some of these effects are consistent with a stress response (Hargreaves 2000). However, due to the complexity and variability of experimental design, it is difficult to extrapolate from such studies what should be recommended for rats in terms of cage size and spatial density.

(v) There is substantial evidence that the shape, size, structure, fittings and the overall design of housing influences biological variables and needs to be taken into account in any experimental design (Clough 1982; Fitzmaurice 1988).

2.2 Cage Floor Area

Principles

(i) Determination of minimum floor space requirements for rats may be guided by the basic behavioural and physiological needs of rats. These include eating, drinking, social interaction (including playing and grooming), resting, defaecating and urinating. Rats tend to compartmentalise their living areas for these different activities (Weiss, Ernst and Schick 1982; Novakova and Babicky 1977; Anzaldo, Harrison, Riskowski et al 1994). In terms of physical movements, rats should be able to be able to turn freely without twisting their heads and bodies, walk at least a few steps, stand, stretch upright and play. They also should have room to shelter and rest. The floor area should ensure that no part of a rat's body is unavoidably distorted by contact with the cage in any of the postures that rats are shown normally to adopt (Lawlor 1987).

(ii) Preference tests for cage size have shown that rats prefer substantially larger cages than standard sizes (Weiss, Ernst and Schick 1982; Patterson-Kane 2002). In the study by Patterson-Kane 2002, it was shown that male and female rats showed a preference for a larger cage (1620 cm^2 versus 540 cm^2) whether tested singly or in the presence of 4 cage mates. In a paper by Scharmann 1991, the adequacy of a cage sized 900cm^2 to house 4 rats of 200-250gm was compared with a cage sized 1,800cm^2. It was concluded that it was doubtful if the smaller cage provided enough space for exercise, and in particular for play.

(iii) Factors other than floor area may influence how rats use a floor space - for example, open space in bright light conditions will be avoided (Matsuo and Tsuji 1988). In cages where the same floor area is available, rats have been shown to prefer cages with vertical partitions, forming an inner chamber, over cages with horizontal partitions (Anzaldo, Harrison, Riskowski et al 1994). In this study, the rats used the inner chamber, created by the vertical partitions, for resting. A study by Foulkes 2004 found that rats did not benefit

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from larger (1088cm^2) versus smaller (432cm^2) cages as assessed by stress responses, unless the cages had enrichment items (tube shelter and gnawing / carrying item).

(iv) Guidelines on space requirements commonly relate requirements for space to the weight of the animal (Hackbarth, Bohnet and Tsai 1999). However, other factors need to be taken into consideration such as the stage of the breeding cycle, whether rats are nesting and the age of the rats. A range of behaviours (such as play, sexual activity and sleep) can be affected by the space available (Klinger and Kemble 1985; Saito, Motomura, Taniguchi et al 1996; Kleinlogel 1978). Juvenile rats need space in order to express play behaviour, and play is facilitated by increasing the available space (up to approximately 2,400cm^2) (Klinger and Kemble 1985). In addition juvenile rats have been shown to be more sensitive than older rats to limitations on space (cages 408 – 780cm^2 versus 1080 – 2160cm^2) as assessed by measures of anxiety (Arakawa 2005). Juveniles therefore need more space, relative to their weight, than adult rats.

(v) In addition to body weight and other factors, the body size (for example, length from nose to tail tip) and shape of rats need to be taken into account in determining their requirements for floor area. The relationship between size and weight varies with factors including age, sex and strain (Lawlor 1987).

(vi) In-cage shelters (see 2.9 In-Cage Shelters) are highly desirable additions to rat housing. The dimensions of the floor area must be sufficient to accommodate such furnishings without negatively impacting on rat behaviours because of reduced floor space or restricted access to areas of the cage. Adding in-cage shelters has the contradictory effect of taking away from the floor space but adding space in the vertical dimension.

(vii) To allow mature rats to adopt species typical stances and carry out behavioural activities, Lawlor 2002 has advocated that a living area needs to measure at least 35 (D) x 25 (W) x 18 (H) cm (875 cm^2 floor area) for the smallest females and 50 (D) x 30 (W) x 30 (H) cm (1,500cm ^{2} floor area) for the largest males.

Recommendations

2.2.1 As a guide, based on the information from Scharmann 1991, Patterson-Kane 2002 and Koolhaas 1999, the minimum floor area for a group of up to 5 rats of up to 250-300gm body weight should be 1,500cm^2 and preferably 1,800cm^2. For larger rats, group size should be decreased or cage floor area increased, on the basis that as rats grow, while play behaviour decreases, cage floor area must accommodate other behaviours including social interaction.

2.2.2 As a guide, for a nursing mother and litter (up to weaning at about 21 days), the floor area should be a minimum of 1,500cm^2.

2.2.3 As a guide, for juvenile rats (from weaning to about 50 days), for a maximum group of 12 juveniles, the floor area should be a minimum of 2,000cm^2.

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2.3 Cage Height

Principles

(i) Part of the normal behavioural repertoire of rats is to stand on their hind legs and stretch upright (Buttner 1993). The base of the tail is used as a stabilising tripod and the forepaws may be rested on a firm surface, allowing the rat to stand on tiptoe (Lawlor 2002). The maximum height achieved by rats during upright standing is about 26 - 30cm (Buttner 1993; Lawlor 2002). In a study by Buttner 1993, rats given additional height up to 30cm used this full height on occasion for upright standing.

(ii) Although the ability to rear up is important for rats, they have been shown to prefer a low, dark cage over a high cage, which is most likely related to their desire for shelter. A cage with a variable height may therefore meet rats' requirements for shelter and for rearing (Blom 1993).

(iii) Although rats stand on their hind legs, they do not do this for prolonged periods (Buttner 1993). In a study by Mihara and Hirano 1998, juvenile rats forced to stand on their hind legs for periods totalling 2 hours per day (to reach for food)) developed bony and cartilaginous damage of the femoral heads.

Rats exhibiting stretching and climbing behaviours. Note that the "low top" cage on the left would not allow the rat to fully stretch upright, whereas the "high top" cage on the right accommodates this behaviour. (Photos courtesy of Darek Figa)

Recommendations

2.3.1 Ideally the height of cages should allow rats to stand on their hind legs and stretch up fully. This height does not need to be provided over the entire area of the cage.

2.3.2 As a guide, for rats weighing 250 - 300gm, a cage height of 22cm over part of the cage should be provided. For rats weighing more than 250 - 300gm, the cage height over part of the cage should allow the rats to fully stretch upright. However, it is recognised that currently available cages (with maximum heights of around 22cm - 24cm) are unlikely to accommodate this.

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2.3.3 Where cages are fitted with platforms or in-cage shelters, the distance between the top of the platform or in-cage shelter and the top of the cage should be at least 8cm to allow rats to climb onto the top of the platform or in-cage shelter.

2.3.4 While cage height (over part of the cage) should allow for upright standing behaviour, food and water should be accessible at a level that allows rats (especially juvenile rats) to sit while eating and drinking, to avoid bony and cartilaginous damage.

2.4 Cage Shape

Principles

(i) Cage shapes have been investigated in a number of studies and square cage enclosures are abandoned by rats when given the option of occupying a rectangular cage, even if the square and the rectangular cages have the same total floor space. Preferences by rats are for rectangular cages which allow them closer contact with, and presumably security from, walls (Weiss, Ernst and Schick 1982).

Recommendation

2.4.1 Rectangular rather than square cages should be provided.

2.5 Cage Materials

Principles

(i) The design, construction and management of a rat's immediate enclosure will determine to a large extent how environmental factors, such as temperature, light levels, humidity and air quality impact on the rat (Rose 1996).

(ii) Most rat cages today are solid tubs made of plastics such as polypropylene (opaque) or polycarbonate, polysulphone and polyetherimide (transparent), with wire mesh tops (Hargreaves 2000).

(iii) Opaque cages have the advantage of filtering out harmful glare and allowing rats to hide from humans and neighbouring rats. They have the disadvantages of impeding the observation of rats from outside the cage, restricting rats' vision of activities outside the cage (including that of humans and other rats) and blocking the passage of light, resulting in different light levels in boxes at different levels on cage racks.

(iv) Transparent cages have the advantage of allowing observation of rats from outside the cage. They have the disadvantage of not allowing rats to hide from humans and neighbouring rats. Weiss and Taylor 1985 found that rats exhibited a strong preference for a cage where the rear wall was painted black (versus a fully transparent cage).

(v) Heat is well preserved in solid plastic tubs (such as polypropylene, polycarbonate,) .

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(vi) Cage tops / lids are usually made of stainless steel mesh. The use of "high top" wire mesh lids creates additional height as well as wall areas through which rats can see outside activities and neighbouring rats (whilst still allowing retreat behind the opaque walls of the tub). The use of such "high-top" wire mesh lids creates cage walls that are partially solid and partially open. This facilitates ventilation within the cage.

Recommendations

2.5.1 Rat cages should be made from plastic (for example polypropylene, polycarbonate, polysulphone, polyetherimide) floors and walls ("shoebox" or "tub") with wire mesh tops (unless special purpose cages such as filter top cages or individually ventilated cages are required).

2.5.2 Where transparent plastics are used for cage "tubs", particular attention should be paid to providing rats with shelters to allow them to withdraw from light and activities outside their cage.

2.5.3 The need to monitor rats by observation (the intensity of which will be dictated by the type of project), and the disturbances to rats that may occur in doing this in opaque cages with opaque shelters, needs to be balanced against preferences for opaque walls by rats.

2.5.4 Rat cages should be fitted with "high top" wire mesh lids (on solid sided walls) which enable rats to stretch upright and which facilitate interaction by rats with their surrounding environment (via visual and olfactory inputs). The "high top" area does not need to extend over the entire roof of the cage.

2.6 Cage Flooring

Principles

(i) When rats are given a choice between solid or mesh floors, the overwhelming majority of rats will choose solid floors especially when resting (Manser, Morris and Broom 1995). In addition to showing a preference for solid floors in preference tests, rats have been shown to be prepared to make considerable efforts to reach solid floors to rest (lifting up to 83% of their body weight) (Manser, Elliott, Morris et al 1996).

(ii) Housing rats on wire mesh floors causes neuropathy of the hind limbs. The changes are seen within one week of rats being exposed to wire mesh floors and result in functional and structural changes to the innervation of the hind feet including increased sensitivity of the feet to touch (Mizisin, Kalichman, Garrett et al 1998). The problem is exacerbated the longer a rat is kept on such flooring and the heavier it is. Diabetic rats are more susceptible and may develop neuropathies earlier (Zochodne, Murray, van der Sloot et al 1995). Housing rats on wire-mesh floors also interferes with normal sensorimotor gating mechanisms (neurological controls associated with locomotion) (Weiss, Feldon and Domeney 1999).

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(iii) Rats housed on wire mesh floors with large spaces in the mesh (11mm x 35mm) may develop muscle damage. Rats on such mesh may have to expend effort trying to maintain their balance and keep their feet from slipping through the gaps in the mesh (Fröhlich, Walma and Souverijn 1981).

(iv) In a study comparing rats housed on wire mesh floors versus those housed on solid floors with bedding, the rats housed on the solid floors were less physically active, which was suggested to be a reflection of their relaxed emotional state. In addition to increased movement, the rats on the wire mesh floor also ate significantly more, with no difference in weight gains, which were postulated to be effects of stress (Rock, Landi, Hughes et al 1997).

(v) Because wire mesh floors are open they allow dissipation of heat from the bodies of rats and may thus influence rats' thermoregulatory responses in order for them to maintain body temperature (Brunner, Dipiro and Feldmann 1993).

Recommendations

2.6.1 Solid floors should be provided for rat caging.

2.6.2 Wire mesh floors should not be used for rat caging unless with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to use such flooring. In such cases, a solid floor section and nesting material should preferably be provided. The size of the mesh gaps should not exceed 11mm x 11mm. (See also 3.3 Metabolism Cages)

2.7 Bedding

Principles

(i) Ideally bedding should be free of dust, microbial, parasitic, or chemical contaminants, non-traumatic, moisture absorbent and ammonia binding (Potgieter 1993; Kraft 1980). Good bedding material facilitates behavioural thermoregulation. In addition it is desirable for it to be cheap, readily available and easy to use and dispose of.

(ii) Rats have been shown to prefer bedding of larger particle size (wood shavings) over sawdust (Blom et al 1996).

(iii) Deep bedding may provide opportunities for digging/burrowing behaviour.

(iv) Bedding can produce aeroallergens but this problem tends to be confined to conditions of extremely high stocking density (30-60 rats per cage) with materials other than wood-based bedding (Taylor, Gordon and Tee 1994). Bedding also can be a source of airborne bacteria, fungi and endotoxins with the dust being an important factor in the level of air contamination (Kaliste, Linnainmaa, Meklin et al 2004).

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(v) The type of bedding (as well as the frequency of bedding changes) is a major factor in influencing the levels of ammonia present in cages (Rose 1996). Materials used for laboratory animal bedding may have ureolytic properties which contribute to elevated ammonia levels. With the exception of some hardwoods, these can be deactivated by autoclaving (Gale and Smith 1981).

(vi) Some bedding materials contain aromatic oils which, being volatile organic compounds, can induce changes in the hepatic enzyme systems involved in drug metabolism (Vesell, (Lang, White et al 1973; Weichbrod, Cisar, Miller et al 1988; Buddaraju and van Dyke 2003) and may result in prolonged action of drugs such as anaesthetics (Ferguson 1966). These effects may last for weeks after rats have been removed from such bedding (Davey, Fawcett, Lee et al 2003) Also, decreased growth and increased mortality rates have been found when rat pups are raised on cedar-wood bedding, possibly due to the aromatic oils (Burkhart and Robinson 1978). Pelkonen and Hanninen 1997 examined the cytotoxic and enzyme inducing effects of a variety of types of bedding from different parts of the world. Pine shavings beddings were generally found to be highly cytotoxic (although the least cytotoxic of these was from Australia). Extracts of corn-cob, rice hulls and straws were found to be practically non-toxic. A paper sample from Australia (telephone book strips) was also non-toxic compared to woods, although it showed high enzyme inducing activity. In a study by Burn et al 2006, aspen chips, although inert compared with pine and other woods were associated with higher sneezing rates, worse interstitial pneumonia and higher weight gain in rats than a compressed paper bedding. The cause of this, although not related to ammonia levels, was not able to be identified (Burn CC pers comm)

Recommendations

2.7.1 Bedding should be provided in rat cages and should be in sufficient quantity to cover the whole floor. The depth of bedding required will vary with factors such as the type of bedding used, the number of rats in the cage and the frequency of cleaning. As a guide, the depth of bedding should be at least a minimum of 2 cm.

2.7.2 Ideally bedding should be free of dust, microbial, parasitic, or chemical contaminants, non-traumatic, moisture absorbent and ammonia binding. The properties of bedding provided should also include that its particles can be manipulated and/or that it is suitable for digging / burrowing.

2.7.3 In choosing bedding, the potential for bedding types to induce hepatic enzymes needs to be taken into consideration.

2.8 Nesting Material

Principles

(i) Rats of most strains, whether wild or captive, build nests routinely. This behaviour has been observed in young and old, male (Jegstrup, Vestergaard, Vach et al 2005) and female rats and is thus not just relevant to adult females or dependent on pregnancy. There is some evidence to suggest that laboratory rats make better use of nesting material, in terms of better nest building and less eating of nesting material, if provided with the material from

birth (Van Loo and Baumans 2004). The provision of nesting material assists rats in manipulating their microenvironment.

(ii)Rats commonly do not build well-shaped nests (Manser, Broom, Overend et al 1998a). However, in a study by Jegstrup, Vestergaard, Vach et al (2005) where in-cage shelters were provided, rats (male) built complex nests within the shelters. In this study, the use of nesting material by the rats varied with the stain of rat, the type of nesting material and the way the nesting material was presented (eg top of cage lid versus within the cage).

(iii) Rats' ability to manipulate and move objects is best catered for by providing them with loose, light materials on top of the bedding. Rats show a marked preference for coarse materials for nest building and show a preference for long paper strips (Manser, Broom, Overend et al 1998a; Manser, Broom, Overend et al 1998b), straw (Jegstrup, Vestergaard, Vach et al 2005) or woodwool. Some lighter materials, such as thick paper or wheat husks, offer the additional opportunity for burrowing.

(iv) The use of nesting material is linked to the survival of pups. According to one study, woodwool was superior as a nesting material and led to a significantly higher survival of pups than those raised in paper tissue or without any nesting material (Norris and Adams 1976). However, care should be taken with the type of nesting material provided - for example, pups may suck on cotton wool and subsequently choke; some pulped cotton fibre nesting materials may separate into strands that wind around pups' legs.

Recommendations

2.8.1 All rats should be provided with nesting material in addition to bedding material.

2.8.2 Nesting material should be loose, manipulable and light enough to be carried. Suitable materials include shredded paper, straw and woodwool.

2.8.3 The way in which nesting materials are provided (eg top of cage lid versus within the cage) should take into account stain-specific differences in the use of materials, depending on the site where they are provided.

2.9 In-Cage Shelters

Principles

(i) When rats are placed in an outdoor cage with an earthen floor they will dig a burrow almost instantly. Rats, like all rodents, have a strong need for hiding (Boice 1977). Rats have been shown to prefer cages with shelters to barren cages (Townsend 1997; Manser Broom, Overend et al 1998b; Patterson-Kane 2003). They show more exploratory behaviour and are less fearful to handle when housed in cages with shelters rather than in barren cages (Townsend 1997).

(ii) Rats' preferences for in-cage shelters can be influenced by strain, age, sex and housing conditions. In a study by Galef and Sorge 2000, some male rats used PVC tubes rarely or

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only at night while juvenile and female rats used them extensively, regardless of the time of day.

(iii) Rats have been shown to choose to be in opaque as opposed to transparent shelters (even when light levels in both shelters are the same, and the same as the light levels outside the shelters), demonstrating a preference for areas where they are not only physically protected but where they cannot be seen (Ambrogi Lorenzini, Baldi, Bucherelli et al 1993; Patterson-Kane 2003).

(iv) In-cage shelters have several functions and can be used for a variety of activities that are part of the rat's natural repertoire:

* They allow withdrawal from light.
* They give a choice of microclimates which aid in rats' thermoregulation (it is darker, more humid and usually warmer in a shelter).
* They provide a means of escape from aggressive social interactions and offer a degree of control to the rats.
* They better satisfy the thigmotactic (wall hugging) aspects of rat behaviour than one large cage.
* They may provide an additional structure as a climbing platform, enhancing the rats' ability to use vertical space.
* They may facilitate the use of nesting material ((Jegstrup, Vestergaard, Vach et al 2005).

(v) Rats, when given a choice, choose housing offering maximum hiding ability. In studies that compared partitions to nest boxes, nest boxes were preferred (Manser, Broom et al 1998a).

(vi) An enclosed, opaque thermoplastic nest box with a small entrance hole appears to be the preferred option for a shelter but other forms of shelter are possible (Patterson-Kane 2003; Manser, Broom, Overend et al 1998a). For instance, just darkening a wall area by placing some black self-adhesive plastic over the outside of part of the cage (for transparent cages) and placing a roof over the area that has been darkened, has been found to be effective as an area for sheltering. Partitions do not have the same benefits as providing a roofed section but they can assist in allowing an individual to escape from stressful social situations.

(vii) Rats will attempt to chew items, including shelters, placed within their cage. Using materials (such as cardboard or polypropylene) that can be chewed by rats has the advantage of allowing rats to perform this gnawing behaviour but the disadvantage that the shelters will be damaged. In one study, in-cage shelters made from old polypropylene mouse boxes, upturned and with one end cut out, were used. The rats chewed these, but no problems attributable to this were recorded over a period of more than a year (Townsend 1997).

(viii) Given the opportunity, rats will make use of space in the vertical as well as horizontal plane. In-cage shelters made from slippery materials may prevent rats from making use of the vertical space by making it difficult for them to climb onto the roof of the shelter.

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Shelters with flat tops enable rats to use the vertical space to climb and stand on top of the shelters.

(ix) Suitable sizes for in-cage shelters quoted in the literature are: 25cm (D) x 17cm (W) x 12cm (H) (Manser, Broom, Overend et al 1998a), and 33cm (D) x 15cm (W) x 13cm (H) (Townsend 1997).

Rats make use of in-cage shelters (photos courtesy of David Morton)

Recommendations

2.9.1 Rats should be provided with a shelter within their cage.

2.9.2 In-cage shelters should ideally have solid, opaque sides and roof that allow withdrawal from the light (and from other rats) and should be constructed so that rats are able to climb onto the roof of the shelter.

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2.9.3 Where in-cage shelters are made of chewable material, it should be ensured that the material is not toxic to rats.

2.9.4 The minimum space between the roof of the shelter and the top of the cage should be 8cm to allow for rats climbing onto the roof of the shelter.

2.10 Pens

Principles

(i) Rat pens and enlarged cages, that allow for large areas in which rats can move, both in horizontal and vertical planes, have been described (Spangenberg, Augustsson, Dahlborn, Essen-Gustavsson and Cvek 2005; Sorensen, Ottesen and Hansen 2004; Hurst, Barnard, Hare et al 1996). In the study by Spangenberg et al, male rats in groups of 8 in pens measuring 3.15m^2 were shown to have physiological parameters reflecting increased physical fitness (eg increased muscle strength and endurance), over individually housed rats in standard cages. It was concluded that the large pen provided an environment that stimulated physical activity and more varied behaviour. (However, it should be noted that the use of rats housed individually may have introduced confounding variables influencing the findings of this study).

An enlarged cage with shelters, ledges and a ramp, allowing for movement in both horizontal and vertical directions

Recommendations

2.10.1 The use of pens and enlarged cages, with furnishings such as shelters, ledges and ramps, may be considered as a viable alternative to conventional caging for rats.

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3. Rat Care and Management

3.1 The Social Environment

Principles

(i) Rats are well adapted to living in groups (Lore and Flannery 1977).

(ii) Social contact with conspecifics is important to the rat (Patterson-Kane, Hunt and Harper 2002, Hurst, Barnard, Nevison et al 1998. Successful group housing is not just a matter of stocking density but of the combination of individuals. (Hurst, Barnard, Tolladay et al 1999).

(iii) Within the cage environment, social interaction is influenced by the space available per rat (spatial density) and the numbers of rats in the group (social density). Crowding can result from a decrease in spatial density and/or an increase in social density.

(iv) Confined environments (inherent in rat caging) have a negative effect on the behaviour of rats by frustrating social rules of conduct (Hurst, Barnard, Tolladay et al 1999).

(v) The impact of crowding parameters on rats appears to differ between males and females, with one study indicating that males show a greater stress response to spatial crowding, and females show a similar response to spatial and social crowding (and a maximal stress response to single housing) (Brown and Grunberg 1995). Other studies in male rats, where available space was decreased and number of rats per group increased, have shown significant effects on taste preferences, body weight gain and feed and water intake, and a variable effect on the measures of stress responses (Scalera 1992; Chaouloff and Zamfir 1993); however exploratory behaviour was not affected (Chaouloff and Zamfir 1993).

(vi) Lawlor 1990 has stated that group size generally has a greater effect on the well-being of rats than cage size (ie social density versus spatial density). In studies conducted by Lawlor 1987, when the size of the group of rats was 12 or 24 at low spatial densities (low space per rat: 67-133cm^2 per rat), growth and health were impaired and the rats were more nervous to handle (although their general appearance erroneously suggested that they were normal). Rats in groups of 6 at intermediate spatial densities showed similar, but less marked abnormalities. Rats kept from weaning in groups of 3 at high spatial densities (high space per rat: 500-1000cm^2 per rat) did not exhibit these abnormalities. Lawlor concluded that a group of 3 rats, especially if caged together before maturity, can establish and maintain orderly social relationships while a group of 6 or more cannot (Lawlor 1987). Klir et al 1984 looked at the effects of housing male rats in groups of 2, 3, 4, 6 and 8 and concluded that housing rats in groups of 3 or 4 per cage had the least effects on physiological changes. In a study using male rats in groups of 1,2 and 4, Sharp et al 2002 found that stress-like responses were significantly reduced when rats were housed in groups of 4 compared with rats housed alone. Housing the rats in pairs did not always reduce the stress-like responses to the same degree as housing 4 per cage. Patterson-Kane, Hunt and Harper 2004 conducted a study that showed that female rats preferred a group size of 6

(versus 1,2,4 and 12) when tested over 90 minute sessions. Hurst et al 1999 looked at the effect of housing both male and female rats in groups of 1, 3, 5, and 8 on behavioural and pathophysiological indices of stress and found that group size had a limited long term effect on behaviour and did not affect pathophysiological responses. In this study, a high level of individual variation was found, which may be due to the fact that these rats were housed in wire cages

(vii) Lawlor 1987 has stated that in juvenile rats, holding littermates together in groups of up to 12 after weaning until approaching sexual maturity has been found to cause no marked disadvantages (for example in growth and ability to carry out normal behaviours), providing the rats have sufficient space. (See 2.2 Cage Floor Area).

(viii) Peters et al 1981 found group sizes influenced toxicological responses in rats housed in groups of 1, 2 or 3 and showed significant differences between males and females, in that group numbers had a more significant effect on males and isolation had a more significant effect on females.

(ix) Husbandry procedures such as handling can disturb rats' abilities to recognise cage mates (social memory). After such procedures, the behaviour of rats should be monitored to ensure that any disturbance does not result in agonistic and potentially injurious behaviour (Burman and Mendl 2004). It has been shown that juvenile rats can successfully recognise familiar cage mates after a period of separation of at least 48 hours. Successful recognition was not demonstrated at 96 hours (Burman and Mendl 2006).

(x) Young rats, and sometimes even older rats, engage in play behaviour which includes leaping, chasing and scuffling (Scharmann 1991). In monitoring rats for "real fighting" versus "play fighting" indicators including the following can be used:

* target of contact – during "play fighting', snout or oral contact is directed at the opponent's nape of the neck, whereas in "real fighting" contact is directed at the opponent's rump;
* hard bites – bite wounds may be sustained in "real fighting";
* raised hairs (piloerection) in "real fighting" (Pellis and Pellis 1987);
* 50 kHz (ultrasonic) vocalisations during play (Portfors 2007).

Recommendations

3.1.1 As a guide, optimal numbers for groups of adult rats is probably up to 4 individuals. In deciding optimal group sizes, factors such as differences between individuals, strain, sex and cage size should be taken into account.

3.1.2 Juvenile stock rats may be housed in groups of up to 12 rats (preferably as litter mates) until approaching sexual maturity (around 50 days of age) (Lawlor 1987).

3.1.3 Ideally rat groups should be made up of litter mates of the same sex.

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3.1.4 Rats should be grouped with each other before they reach puberty to avoid or minimise problems of aggression between unfamiliar individuals (Hurst, Barnard, Nevison et al 1999).

3.1.5 The disruption of established social groups should be avoided.

3.1.6 When removing rats from, and reintroducing them to, cage mates, it should be aimed to keep the period of separation to 48 hours or less (to take advantage of social memory).

3.1.7 Where possible, adult males from different groups should not be placed in the same cage.

3.1.8 Where it is necessary to mix unfamiliar adult males, they should be exposed to each other before they are mixed together. This can be achieved by placing the newcomer into an adjoining cage with visual and olfactory contact with the other male.

3.1.9 Shelters should be provided within cages to enable rats to hide in case of conflict.

3.1.10 Groups of rats should be monitored to ensure social stability as well as the detection of behavioural and physiological abnormalities. In monitoring rats for "real fighting" versus "play fighting" indicators during skirmishing such as the target of contact (rump versus the nape of the neck), hard biting and raised hairs (piloerection) can be used (as well as measurement of the frequency of ultrasonic vocalisations).

3.2 Isolation / Individual Housing

Principles

(i) There are many reports in the literature of the effects of "isolation" on behavioural and physiological measures in rats where "isolation stress" is used as a treatment variable. However, in most circumstances, rats are housed individually but are not completely isolated, in that they maintain olfactory, auditory and visual contact with conspecifics (Brain and Benton 1979). Thus, data need to be interpreted carefully, as most reports refer to social rather than physical isolation.

(ii) Although the notion of "isolation stress" in the rat has been challenged (Brain and Benton 1979; Holson, Scallet, Ali and Turner 1991), individual housing of rats (ie social isolation), is associated with a range of behavioural and physiological changes, some of which indicate a stress response.

(iii) The effects of individual housing will vary with the period of isolation, age, sex and strain and the prior housing history of the individual.

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(iv) Some of the effects of individual housing can be ameliorated by ensuring visual, auditory and olfactory contact with other rats (Hurst, Barnard, Nevison et al 1997; ibid 1998).

(v) The behavioural and physiological effects of individual housing can be reversed when animals are returned to group housing (Hatch, Wiberg, Balazs and Grice 1963; Gentsch, Lichsteiner, Frischknecht et al 1988) or ameliorated by handling (Gardiner and Bennett 1977;Gentsch, Lichsteiner, Frischknecht et al 1988; Holson, Scallet, Ali and Turner 1991; Reboucas and Schmidek 1997).

(vi) Environmental enrichment by provision of toys, reduces baseline levels of ACTH and corticosterone in both male and female rats housed individually, and lowers the ACTH response to a mild stressor in female rats (Belz, Kennell, Czambel et al 2003).

(vii) Reported behavioural and physiological consequences of individual housing in rats include:

* Behavioural changes consistent with social deprivation (such as reduced mobility, increased tail chasing and self-grooming) (Hurst, Barnard, Nevison et al 1997),
* Altered reactivity to a novel environment when compared with group housed animals, but this response is seen only in some aspects of behaviour (Gentsch, Lichsteiner and Feer 1981; Hall, Humby, Wilkinson and Robbins 1997a) and is dependent upon the aversiveness of the test environment eg. light conditions (Hall, Humby, Wilkinson and Robbins 1997b),
* When reared in isolation from weaning, increased aggression when introduced to other rats in an aversive environment (Wongwitdecha and Marsden 1996),
* Modulation of the daily rhythms of hypothalamic catecholamines, their metabolites and circulating hormones (Greco, Gambardella, Sticchi et al 1992; Gambardella, Greco, Sticchi et al 1994),
* Increased levels of circulating corticosterone and prolactin were reported in male rats (Gambardella, Greco, Sticchi et al 1994), but Brown and Grunberg 1995 when comparing individual versus crowded housing conditions, found an increase in corticosterone levels in female but not males when housed individually,
* Increase in blood pressure and heart rate (Carlier, Crine, Yerna and Rorive 1988; Gardiner and Bennett 1977; Lawson, Churchill and Churchill 2000; Sharp, Zammit, Azar and Lawson 2003), myocardial hypertrophy (Carlier, Crine, Yerna and Rorive, 1988) and increased responsiveness to noradrenaline in arterial strips (Parra, Funetes and Alsasua 1994),
* Variations in biochemical (Perez, Canal, Dominguez et al 1997) and immunological (Baldwin, Wilcox and Baylosis 1995) measurements,
* A deficit in sensorimotor gating (Krebs-Thompson, Giracello, Solis and Geyer 2001) which is attenuated by handling, and
* The development of an abnormal gait in animals housed in social isolation from weaning (Roberts, Clarke and Greene 2001).

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(viii) There are equivocal results in studies involving measures of stress in response to individual housing and the diversity of test conditions in the different studies is likely to be a major factor contributing to these equivocal findings.

Recommendations

3.2.1 Rats should not be housed individually unless with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to house rats in this way. In such cases, rats should be able to be in visual, auditory and olfactory contact with other rats.

3.3 Metabolism Cages

Principles

(i) When metabolism cages are used to house rats individually, the degree of physical isolation is greater than from individual housing in standard cages, in that the design of metabolism cages will restrict their exposure to olfactory, auditory and visual contact with other rats. Further, they will be housed on a wire mesh floor (see section 2.6 Cage Flooring). Thus the potential impact on the well-being of the rats is greater and there are fewer options to ameliorate these effects.

(ii) When rats are housed in metabolism cages there is a decrease in food and water intake, urine output and creatinine clearance (Damon, Eidson, Hobbs and Hahn 1986; Vadiei, Berens and Luke 1990) and an increase in urinary excretion of corticosterone and aldosterone (Gomez-Sanchez and Gomez-Sanchez 1991).

(iii) A minimum acclimatisation period of 4 days is recommended for rats to recover from the effects of being placed in metabolism cages.

(iv) A significant reduction in the kinetics of drug excretion is seen when rats are housed in metabolism cages for 8 days (Brunner, Dipiro and Feldman 1993).

(v) Behavioural and metabolic effects of housing rats in metabolism cages varies with age. In both young (3 months) and older (12 months) rats, there is an initial increase in urinary excretion of norepinephrine which is sustained in the older animals but returns to normal in the young animals (Gil, Aguirre, Lemoine et al 1999).

(vi) Pregnant rats housed in metabolism cages decrease food intake and lose weight, and there is an increase in the incidence of skeletal malformations in the foetuses (Bosque, Domingo and Corbella 1994).

(vii) A study has been reported in which metabolism cages were enriched with an area of solid floor or with an area of solid floor and a nest box. The rats frequently used the enrichment and the enrichment had no significant effects on food and water intake, faeces production or urine creatinine (Bolder and Blom (unpublished)).

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Recommendations

3.3.1 Rats should not be housed in metabolism cages unless with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to house rats in this way. In such cases, rats should be able to be in visual, auditory and olfactory contact with other rats as far as possible.

3.3.2 Rats should be acclimatised to the metabolism cage before studies commence.

3.3.3 Where metabolism cages have to be used, consideration should be given to enriching the cages (for example with an area of solid floor and a nest box), providing this does not interfere with the study.

3.4 Individually Ventilated Cages

Principles

(i) There is limited information in the literature on individually ventilated ages related to the welfare of rats. Possible concerns related to individually ventilated cages include the limitations on size (and hence limitations on the provision of physical and social enrichment), the use of transparent walls, and possible ultrasound from air being forced in and out (Sherwin C pers comm) (see also 4.5 Air Quality and Ventilation and 4.6 Sound and Vibrations).

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Recommendations

3.4.1 Principles and recommendations related to housing rats in conventional cages apply to housing rats in individually ventilated cages.

3.5 Effects of Handling and Human Activity

Principles

(i) In both animal holding facilities and the laboratory it is inevitable that rats come into contact with humans either directly when they are handled or indirectly when they are exposed to human activity. In both situations, interactions with humans elicit physiological and behavioural responses which have implications for animal welfare and the validity of data collection (Claassen 1994).

(ii) Physiological and behavioural changes are seen not only when rats are handled during experimental procedures (Gartner, Buttner, Dohler et al 1980), or in routine animal care, such as cage cleaning (Duke, Zammit and Lawson 2001; Saibaba, Sales, Stodulski et al 1996), but also when animals are moved into a new facility (Dymsza, Miller, Maloney and Foster 1963; Fortmeyer 1974; Landi, Bowman and Campbell 1988), to a different room (File and Peet 1980; Morato and Brandao 1996; Tabata, Kitamura and Nagamatsu 1998) or moved within a room, for example from a cage rack to a work bench (Gartner, Buttner, Dohler et al 1980).

(iii) Familiarity with the room and their home cage affects measures of locomotor activity (Galani, Duconseille, Bildstein and Cassel 2001), and removing animals from a familiar

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cage affects drug kinetics (Hashimoto, Kawasaki and Gomita 2000; Sun, Falk, Nguyen and Lau 2000).

(iv) Habituation of rats to the testing room and apparatus attenuates hormone responses (File and Peet 1980; Cooper, Mole, Rehnberg et al 1992), and behavioural indicators in pain studies (Milne and Gamble, 1989; Aloisi, Albonetti and Carli 1994).

(v) Rats also respond to the level of activity in their surroundings. For example, significant fluctuations in urine and faecal output were found during weekends compared with weekdays in rats housed in metabolic cages (van der Touw, Thrower and Olley 1978) and significant differences in the architecture and permeability of the mesenteric microvasculature can be associated with the general level of human activity in the room where rats are housed (Wilson and Baldwin 1998).

(vi) Rats communicate their experience of stress by olfactory and aural cues. Procedures or treatments which elicit physical or emotional stress responses in rats will elicit a stress response in non-treated animals held in the same room. For example, control rats placed in the same room will show the same corticosterone response as adjacent animals which have experienced restraint stress (Pitman, Ottenweller and Nantelson 1988). Similar effects have been shown with stress-induced hyperthermia (Kikusui, Takigami, Takeuchi and Mori 2001), increased heart rate (Sharp, Zammit, Azar and Lawson 2003) and immunological challenge (Fernandes 2000). Further, when a rat is introduced into a new room it will show behavioural evidence of stress, changes which are also seen in the non-transported animals in the same room (de Laat, van Tintelen and Beynen 1989).

(vii) Removing a rat from a cage causes an increase in corticosterone levels in the remaining cage mates (van Bergeijk, van Herck, de Boer et al 1990). This communication of the stress response also results in an effect, related to the order in which rats are removed from their cage, on biological measures (Knott, Hutson and Curzon 1977; Brodin, Rosen, Schott and Brodin 1994).

(viii) Behavioural and physiological changes are seen in rats for several hours following routine cage cleaning (Saibaba, Sales, Stodulscki et al 1995; Duke, Zammit and Lawson 2001). These effects need to be taken into account when scheduling experimental procedures.

(ix) A study by Abou-Ismail et al (in press) compared the effects on rats of husbandry procedures (eg weighing and cage cleaning) carried out during the light phase or the dark phase (in the presence of dim red light) of the light/dark cycle. The results suggested that rats having husbandry procedures carried out during the light phase displayed higher levels of various behavioural, physiological and pathological measures indicative of reduced welfare, such as higher aggression, less sleep, elevated chromodacryorrhoea and lighter thymus glands compared to the ‘dark phase’ rats.

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(x) Significant differences in behavioural and physiological responses can be seen depending on whether or not rats are familiar with the people involved in activities (McCall, Lester and Dolan 1969; Gartner, Buttner, Dohler et al 1980; Dobrakovova, Kvetnansky, Oprsalova and Jezova 1993; Thompson, Brannon and Heck 2003, van Driel and Talling 2005).

(xi) Human interaction with rats by handling has been shown, in some circumstances, where rats are accustomed to such handling, to be rewarding for rats (Davis and Perusse 1988).

(xii) Habituation to handling is associated with a significant reduction in the stress response in serum prolactin, corticosterone and ACTH (Yelvington, Weiss and Ratner 1985; Uphouse, Nemeroff, Mason et al 1982) but not norepinephrine, suggesting that habituation involves the hypothalamic pituitary adrenal axis but not the peripheral sympathetic system (Dobrakovova, Kvetnansky, Oprsalova and Jezova 1993).

(xiii) Systematic, gentle handling can be used to habituate rats to handling and routine procedures. Such habituation can ameliorate the effects of stress associated with human interactions. This will have benefits both for the welfare of the rats as well as for reducing the influence of stress responses on experimental results (for example, Corda, Biggio and Gessa, 1980; Shyu, Mordenti, Nightingale et al 1987).

(xiv) Failure to handle young rats can have varied deleterious effects. Pham et al 1999 state that these effects include: deficits in some learning tasks and exploratory behaviour, hippocampal dysfunction and impaired adrenocortical response when exposed to stressful stimuli.

(xv) Handling, in individuals unaccustomed to it, may cause temporary or long term interference with rats' abilities to recognise cage mates (social memory). Familiarising rats to handling can therefore be used to decrease its disruptive / stressful effects (Burman and Mendl 2004).

(xvi) "Gentling", or habituation to handling, is a process of allowing rats to explore the human carer and accustoming them to being gently stroked and held. Cage mates are initially exposed to a human hand (for example while in their home cage, or confined in a bucket) and allowed to sniff and explore it. The rats are touched if they allow it, and the hand may be gently moved under and over the rats. After one to two days the rats can usually be lifted a few inches in one hand and gently stroked. This procedure is repeated for about 5 minutes once or twice daily over about a week (Hirsjärvi and Valiaho1995). As well as reducing fear reactions to handling, gentling has been shown to reduce fear reactions in rats exposed to novel or fear-inducing situations (Hirsjärvi and Valiaho1995).

(xvii) Handling, when coupled with a negative experience, such as an injection, will not result in habituation (Briese and de Quijada 1970; Stewart and Eikelboom 1981), but will lead to rats developing an anticipatory stressor response with associated hyperthermia which has consequences for metabolic studies (York and Regan 1982).

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(xviii) It may be possible to train rats to accept a procedure, such as oral administration of drugs, without restraint using positive reinforcement, for example, drugs delivered in chocolate after a period of training (Huang-Brown and Guhad 2002).

Recommendations

3.5.1 Steps should be taken to allow rats to become familiar with the people who will be handling them so as to reduce the stress of handling. This should include the process of "gentling" (whereby rats are allowed to explore their handler and are gently stroked and held).

3.5.2 "Gentling" (habituation to handling) of rats should not be linked with (ie neither proceed nor follow) procedures that may cause distress to rats.

3.5.3 Handling rats for routine husbandry should not be linked with (ie neither proceed nor follow) procedures that may cause distress to rats.

3.5.4 To reduce the effect of stress responses on rats and subsequently the effects on data collection, rats should be habituated to their surroundings and to routine procedures.

3.5.5 Handling rats at all times should be done quietly and gently.

3.5.6 Experimental procedures should be scheduled taking into account the potential effects on rats of routine husbandry procedures.

3.5.7 The training and rewarding of rats using positive reinforcement or "treats" should be considered when performing procedures on rats. This is likely to reduce the stress on rats and increase their co-operation.

3.6 Environmental Enrichment

Principles

(i) **"**Environmental enrichment" is a vague term referring to improvements in captive animal environments (Newberry 1995). It has been defined as "a concept which describes how the environments of captive animals can be changed for the benefit of the inhabitants" (Young 2003). It has also been defined as "any measure which promotes expression of natural, species specific behaviours and a decrease in, if not disappearance of, abnormal behaviours" (Brinkman 1996, Shomer 2001).

(ii) The aims of environmental enrichment should be not just to prevent suffering, but to have a positive effect on the physical and psychological well-being of the rat (Morton 1993).

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(iii) Rats held in "standard" cages are in a relatively barren environment (lacking in complexity) over which they have little control. Rats have been shown to have long term preferences for complex environments (Denny 1975). A method has been described for creating a physically complex environment in a standard size (1875cm^2) rat cage. It was shown that access to this enriched cage was highly rewarding for rats (van der Harst, Fermont, Bilstra and Spruijt 2003).

(iv) In a review of enrichment of laboratory caging for rats, Patterson-Kane 2004 concluded that rats demonstrate a high demand for social contact and prefer larger cages and cages with shelters, nesting material and foraging devices. She contended that enrichments such as social contact and shelter should be considered basic husbandry requirements rather than optional improvements.

(v) A study by Joffe et al 1973 showed that rats given a degree of control over their environment (over lighting and food and water delivery) showed less "emotionality", or fearful reactions (as assessed by defaecation), in open field tests, indicating an enhanced ability to deal with novel situations.

(vi) As early as 1947 it was shown that rats exposed to enriched environments were better able to problem solve (in a maze apparatus) (Hebb 1947). Exposing rats to enriched environments results in significant changes to their cerebral anatomy, neurochemistry and behaviour. These changes include an increase in the weight and thickness of the cerebral cortex and significant improvements in problem solving abilities. Based on this kind of evidence, it is proposed that rats raised in enriched environments may be better adapted to environmental variation and hence less reactive to change (Rose 1996). There is evidence of greater effects on learning behaviours when rats are exposed to enriched environments in the pre-weaning rather than the post-weaning phase (Venable, Pinto-Hamuy, Arraztoa et al 1988; Pascual and Figueroa 1996).

(vii) In a study by Patterson-Kane et al 1999, rats housed in enriched environments showed less fearfulness in behavioural tests than rats kept in standard cages or housed singly. It was suggested that keeping rats in cages that make them more sensitive to stressors (standard cages) is equivalent to increasing the stressors, which is detrimental to their welfare. The study also showed that the rats from the enriched environments had improved problem solving abilities, implying that a standard environment may not be sufficiently stimulating for rats' problem solving abilities to develop optimally. Van der Harst et al 2003b, in a study looking at the effects of standard versus enriched housing on rats' sensitivity to rewards, also concluded that rats housed in standard cages are stressed, probably because of an inability to satisfy behavioural needs. Rats have been shown to have significantly lower baseline stress responsive hormones (adrenocorticotrophic hormone and corticosterone) when provided with environmental enrichment (items for nesting and gnawing) compared with barren cages (Belz, Kennell, Czambel et al 2003).

(viii) Elliot and Grunberg 2005 looked at differences between social enrichment and physical enrichment. They found that social enrichment had the greatest effect (over

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physical and no enrichment) on improving cognitive performance (simple information processing) in both male and female rats. Overall the effect of enrichment (social and physical) on improving cognitive performance appeared to be greater for males than females.

(ix) The effects of environmental enrichment strategies are manifest in rats' behavioural and physiological responses These involve, at one end of the scale, reduction of the harmful side-effects of standard caging (such as high levels of corticosterone, weight loss, developmental delays or stereotypy) and at the other end of the scale, adding interest to the rats' daily routines and having benefits for the overall health and wellbeing of rats, prolonging their life and usually also leading to them being better research subjects (Rosenzweig, Bennett and Diamond 1972; Diamond, Rosenzweig, Bennett et al 1972; Por, Bennett and Bondy 1982; Widman and Rosellini 1990; Eskola, Lauhikari, Voipio et al 1999; Galef 1999; Benefiel and Greenough 1998, Belz, Kennell, Czambel et al 2003).

(x) It should be noted that the failure to enrich rats' environments may, by imposing constraints on behaviour and brain development, result in aberrant or maladaptive brain functions, which has implications for the usefulness of these animals for research, and in particular for behavioural neuroscience (Wubel 2001). In a review by Sherwin 2004, it was concluded that the development and responses of rodents in standard cages were often unrepresentative and idiosyncratic, indicating that data are likely to have reduced external validity. Sherwin suggests that animals from standard (barren) cages may be "abnormal" and therefore may not provide valid baseline data.

(xi) Rats have five important groups of natural behaviours that should be allowed expression:

* social interaction;
* chewing/gnawing;
* locomotion (including climbing, exploring and playing);
* resting/hiding; and
* manipulating, carrying and hoarding food and objects.

(xii) The suitability of items for enrichment should critically be assessed to ensure that the strategies improve, and are not detrimental to, rats' welfare. Assessment may include, for example, whether enrichment strategies assist with the expression of any of the above behaviours.

(xiii) Enrichment items need to be assessed for their health risks. Some materials, such as some plastics or galvanised or painted materials, may be dangerous because of their toxicity when chewed.

(xiv) In some of the studies quoted above and that of Zimmermann et al 2001 (which describes the provision of a "near-to-natural" environment), the degree of environmental complexity provided may not be practical for the day to day management of rats. It is,

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however, possible to provide opportunities for rats to express particular behaviours within "standard" cages (see for example van der Harst , Fermont, Bilstra and Spruijt 2003).

(xv) Examples of enrichment items include:
Social interaction:
* See 3.1 The Social Environment

Chewing / gnawing:
* Small block of wood drilled with holes (if the block is small enough, it will be chewed into small pieces and disposed of during cage cleaning before it has a chance to become significantly soiled) (Chmiel and Noonan 1996)
* Branches and softwood sticks (eg tongue depressors) (Patterson-Kane, Hunt and Harper 1999; Scharmann 1991; Johnson, Patterson-Kane and Niel 2004)
* "Kong Toys" and "Nylabones" (Belz, Kennell, Czambel et al 2003)

Locomotion (including climbing, exploring and playing):
* Branches (Patterson-Kane, Hunt and Harper 1999)
* Running wheel (Patterson-Kane, Hunt and Harper 1999)
* Ledges
* Space (in 3 dimensions) (Patterson-Kane, Hunt and Harper 1999; Scharmann 1991)
* Provision (and rotation on a regular basis) of novel objects such as plastic "toys" (Patterson-Kane, Hunt and Harper 1999; Scharmann 1991)

Resting / hiding:
* Nesting boxes (Patterson-Kane, Hunt and Harper 1999; Patterson-Kane 2003)
* Nesting material (Patterson-Kane, Hunt and Harper 1999)
* Ledges

Manipulating, carrying and hoarding food and objects:
* Providing food within the cage that can be picked up and held and hoarded (such as sunflower seeds) (Johnson and Patterson-Kane 2003)
* Cellulose paper and straw on the lid of the cage (Scharmann 1991)

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Seeds provide a source of food which can be manipulated and hoarded. (Photo courtesy Darek Figa)

Rats exhibiting climbing behaviour in a cage with a "mezzanine" level. (Photo and cage design courtesy of Darek Figa)

Recommendations

3.6.1 Rats should be provided with items to enrich their environment. Items that assist rats to perform each of the five following categories of behaviours should be provided:

- *social interaction (see Section 3.1 The Social Environment),*
- *chewing/gnawing,*
- *locomotion (including climbing, exploring and playing),*
- *resting/hiding, and*
- *manipulating, carrying and hoarding food and objects.*

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(See 3.5 (xiv))

3.6.2 When techniques are used in an effort to provide environmental enrichment for rats it is important that the success of the techniques, in terms of improving the rats' welfare, is evaluated.

3.7 Identification

Principles

(i) Clause 4.7.1 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states in part:

Animals must be identifiable, whether individually or in groups. Where possible, animals should be identified by the attachment of a label to the cage, container, pen, yard or paddock where they are kept. Otherwise, identification of individual animals may require a physical mark such as a tattoo, neckband, individual tag, or electronic numbering device such as a microchip……The method chosen should be the most appropriate for the species and project and result in the least pain and distress to the animal.

(ii) Ideally methods of identification should not be painful, not cause adverse reactions, not be uncomfortable and not likely to catch or tear out.

Recommendations

3.7.1 Where it is necessary to individually identify rats, the least invasive method that is compatible with the use of rats should be used.

3.7.2 Non-toxic dyes and permanent markers may be used on the fur and tail. These methods of identification usually need to be replaced every two weeks.

3.7.3 Fur clipping may be used but needs to be carried out frequently.

3.7.4 Subcutaneous microchipping, tattooing and ear notching may be used where permanent identification is necessary. Note there is some transitory pain associated with applying these forms of identification and the use of anaesthesia and/ or analgesia should be considered..

3.7.5 Toe and tail tip amputation are painful procedures and should not be used.

3.8 Food and Water

Principles

(i) Rats need to have food and water provided *ad libitum*. Note that food and water consumption are affected by the social environment (see 3.1 (iv)).

(ii) Food and water should be free of contaminants (Newberne 1975; Lang and Vesell 1976; Tucker1987) and be of a quantity and quality to meet the rat's nutritional needs, taking into account the special needs of pregnant, lactating or growing animals (National Research Council 1995). If possible, the types of food provided should be varied.

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(iii) Rats are nocturnal feeders (Siegel 1961; Wong and Oace 1981). The normal behaviour of rats when eating is to carry a piece of food by their teeth to a suitable spot where they adopt a squatting posture and hold the food in their forepaws to nibble at it (Lawlor 2002).

(iv) Providing items of food within the cage rather than in fixed dispensers can encourage foraging behaviours and allow rats to adopt normal postures for eating.

(v) Ad lib feeding may result in obesity in rats. In a study by Wrightson and Dickson 1999, food hoppers were modified to reduce the area over which food was available. Food was provided ad lib but rats worked harder for their food, enabling them to burn more calories and be significantly lighter than control rats after a period of eight months. However, in a study by Johnson, Patterson-Kane and Neill 2004, rats preferred a device that enabled foraging over other feeding sources including a limited access hopper. The rats that were able to forage showed reduced aggression and were able to search for and manipulate food, but they had significant gains in body weight. This weight gain was thought to be due to rats gaining access to whole pellets and it was postulated that the effects could be mitigated by providing a lower calorie food.

Recommendations

3.8.1 A nutritionally adequate diet should be provided for rats.

3.8.2 Food and water should be provided ad libitum unless special permission has been obtained to vary this regime from the Animal Ethics Committee of the institution.

3.8.3 Variations in the types of food and how it is presented should be provided (for example, commercial pellets, dried sunflower seeds, corn on the cob, fresh vegetables).

3.8.4 Food items should be provided not only in food hoppers but should also be sprinkled onto the cage floor bedding to add interest, foster foraging behaviour and promote normal postures during feeding.

3.8.5 The rat's nocturnal feeding patterns should be taken into account in study design, especially when treatments are given in the diet.

3.9 Monitoring of Rats

Principles

(i) Rats are affected by their living conditions, including their physical environment, their social environment and their interaction with humans. When assessing the responses of rats to their living conditions, assessment of physiological and behavioural parameters are useful. Negative trends in these parameters, such as loss of body weight, failure to reproduce and changed behaviour patterns may indicate that the rats are distressed and failing to cope with their environment.

(ii) Behavioural observations can provide useful cues and early warnings that something is wrong with a rat's state of health and wellbeing. A range of responses may be observed from subtle changes in normal patterns of behaviour to stereotypy (which is a clear sign of a rat's inability to cope with its environment). Excessive grooming, aggression or states of fear are examples of behavioural indicators that a rat is distressed. Abnormal behaviour may manifest itself as an increased reactivity to environmental stimuli, leading to panic reactions, or to an increased passivity or state of depression (Koolhas 1999). Persistent and intense gnawing, as well as short-distance pacing, are typical stereotypic behaviours. The duration and kind of stereotypic behaviour is important when assessing its welfare significance.

(iii) Excessive secretion of porphyrin from the Harderian gland which is behind the eye (chromodacryorrhoea) is an indicator of stress in rats (Canpolat 2003). This can be seen as a red stain around the eyes and muzzle but often as red streaks from the eyes across the back of the head as the animals spread the excess dye by grooming. Mason et al (2004) showed that low level transient Harderian secretions (seen as specks around the nose) can be scored to assess low to moderate levels of stress.

(iv) As rats are nocturnal, the full range of wake-hour behaviours can best be observed at night using minimal light illumination.

(v) Fearfulness can manifest itself in behaviours such as freezing, hesitancy, and long latency in emerging from familiar spaces (Patterson-Kane, Hunt and Harper 1999). Other signs of fearfulness can be intense vocalisations, teeth chattering, fur fluffing, coupled with immediate defecation and urination. Examples of situations that can elicit fearful behaviour include: exposure to an unfamiliar environment, social isolation, exposure to predators and the conduct of painful procedures.

(vi)Abou-Ismail et al 2007 showed that low frequencies of sleep behaviour and low sleep duration in rats (during the light phase of the light/dark cycle) correlated with some indicators of stress (adrenal weight and body weight gain). They postulate that the stress experienced disrupts sleep behaviour and therefore that the monitoring of sleep behaviour may provide a non-invasive indicator of stress and welfare.

(vii) The results of a study by Burman et al 2007 suggested that ultrasonic vocalisation by rats of 22kHz could induce a negative emotional state of increased anxiety in rats hearing the vocalisation, and could therefore be a useful indicator of welfare for rat groups, including both callers and non-calling group mates. Additionally the recording of 50kHz vocalisations (indicating a positive emotional state) could be used to distinguish normal play in juveniles from aggression (Portfors 2007).

(viii) Gnawing and chewing objects other than food is generally considered to be a natural behaviour that can escalate into a stereotypy with chronic stress (Levine and Morley 1982; Reynolds and Kimm 1972). Sorensen et al 2004 have proposed that excessive gnawing may

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be escape related and may be indicative of frustration related to the rats' environment and hence suggestive of reduced welfare.

(ix) Similar to gnawing, grooming serves a number of functions. Increased grooming is seen when rats are stressed, as part of their coping mechanism (van Erp, Kruk, Meelis and Willekens-Bramer 1994; Moyaho and Valencia 2002). In rats which are chronically stressed this excessive grooming can result in hair loss.

(x) As noted in the section on "Effects of Handling and Human Activity" (3.4), rats respond behaviourally and physiologically to unfamiliar people. Consequently, the 'setting' in which behaviour is monitored and assessed is important so as to be able to critically evaluate the impact of housing conditions on the rat's well being.

(xi) The development of objective monitoring systems for animals undergoing research procedures, to assist in the recognition of pain and distress, have been described (Morton and Griffiths 1995, Bate 2003, Roughan and Flecknell 2006).

Recommendations

3.9.1 Welfare monitoring of rats via behavioural observation should be carried out in addition to monitoring for general physical health.

3.9.2 Monitoring should be carried out when a person with whom the rats are familiar is present.

3.9.3 In the monitoring and investigation of heath issues (such as growth rate, reproductive performance and disease) the effects of housing conditions should be taken into account.

4. Environmental Variables

4.1 General

Principles

(i) The goals of good animal care and management should be to keep animals healthy and comfortable and to meet their physiological and behavioural needs. Management of environmental variables such as temperature, humidity and lighting can play a significant role in achieving these ends. Some adverse factors such as noise, flashes, and vibrations may not easily be identified but their absence will contribute significantly to the well-being of rats.

4.2 Light

Principles

(i) Light is an important environmental variable which has the potential to affect the health and behaviour of rats (Schlingmann, De Rijk, Pereboom and Remie 1993a). For the overall well-being of rats and for their basic locomotor needs, it is essential to manage light levels

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well. Room illumination, including the light reaching the actual cages, has important effects on rat well being because day-length, light intensity and spectral quality (wavelength) can affect the functioning of the physiological systems and the behaviour of rats (Belhorn 1980).

4.2.1 Light Intensity and Wavelength

(i) Lighting intensity of various levels has been associated with eye pathology and with causing behaviours in rats indicating aversion. Light intensity can influence factors such as how rats use a living space and their sleeping patterns.

(ii) Rats are nocturnal and the photoreceptors in their eyes are adapted to dim lighting conditions between 1- 40 lux (Vandershuren, Niesink, Spruijt et al 1995). Albino rats lack the pigment melanin that normally protects the eye against high light intensity. These strains have greater light aversion than pigmented strains (Matsuo and Tsuji 1988).

(iii) Tests of avoidance behaviour at different light intensities have shown that albino rats avoid light intensities as low as 25 lux and pigmented rats from as low as 60 lux (Schlingmann, De Rijk, Pereboom et al 1993a). The authors concluded, because the rats were motivated to leave a warm nest to avoid these light intensities, that exposure to these intensities caused distress.

(iv) Exposure to bright (high intensity) light (100 - 200 lux) reduces activity levels in most strains of adult rats, thus affecting normal locomotor behaviour (Matsuo and Tsuji 1988).

(v) In juvenile rats, exposure to bright light (572 lux) suppresses play behaviour which is otherwise displayed regularly when they are housed under dim (low intensity) (0.4 - 1 lux) lighting conditions (Vandershuren, Niesink, Spruijt et al 1995).

(vi) Light levels within the cage influence sleep postures and wall contact by rats. Van Betteray, Vossen and Coenen 1991 found that when exposed to bright (high intensity) light (500 – 600 lux), rats sleep curled up into a ball (nose to tail) and in contact with the cage walls. Under low intensity light (6 - 9 lux) they lie with their head extended, rather than tucked under their body. The lower the light intensity, the less they slept against the cage walls. These authors postulated that wall hugging was a sign of fearfulness and that bright light may provoke fear behaviour.

(vii) The level of light to which rats are exposed will be influenced by the position of the rack in the room, relative to the light source, and the position of the cage within a rack (Kupp, Pinto, Rubin and Griffin 1989). Subtle differences in light levels, for example between the top and bottom levels of a rack, will influence behaviours and can interfere with investigations, such as those looking at the effects of treatments on behaviour (Exner and Clark 1993). Modifications to the top shelf of a rack by providing extra shading helps to shield the lower shelves from light and create a more uniform light level between shelves (Williams and Howell 1983; Schlingmann, De Rijk, Pereboom et al 1993b).

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(viii) Rats undergo some degree of biochemical adaptation if light is a fraction brighter than would be expected in the natural environment (Penn and Anderson 1992).

(ix) Light exposure can be associated with retinal pathology. In albino rats, retinal degeneration develops within 13 weeks of exposure to light intensities as low as 60 lux (Stotzer, Weisse, Knappen and Seitz 1970) and, in these conditions, blindness can occur (Weihe 1976). It should be noted that such light intensities are commonly found within cages. However, exposure to higher light levels for a brief period also will cause permanent retinal damage. A study by Williams, Howard and Williams 1985 showed that albino rats exposed to 133 lux for three days had lost 50% of their retinal rod nuclei as compared to the control group maintained at 3l lux.

(x) The retina of the rat is only partially developed at birth, developing even after rats have opened their eyes (Penn and Williams 1985; Kupp, Pinto, Rubin and Griffin 1987; Penn and Anderson 1992). In rats that have been reared under conditions of relatively high light intensities, the development of the rods in the retina is inhibited. This may later lead to the false assumption that these rats are less sensitive to light when, in fact, this is only the result of damage inflicted on the eyes early in the rats' lives (Semple-Rowland 1987 as cited in Schlingmann, De Rijk, Pereboom et al 1993a).

(xi) Weihe et al 1969 showed that light intensity significantly affects reproductive performance, specifically the number of litters born, the numbers per litter and weight gain during gestation.

(xii) Although rats should not be exposed to high light intensity, operators in animal rooms need to have enough light to perform visual tasks. Schlingmann et al 1993b concluded that 210 lux at working height is sufficient for the health and performance of technicians, but would be the minimum under which they should be expected for work for any length of time.

(xiii) Flickering light at only 80 lux intensity, and for a period of 30 minutes, has been shown to be a potent stressor on albino rats causing significant biochemical changes, comparable to those reported in rats that were stressed by electric shock treatment (Lalitha, Suthanthirarajan and Namasivayam 1988).

(xiv) Although it is commonly believed that rats cannot see red light, some writers assert that rats can detect red light, because the rat eye can absorb some low intensity red light and it also has a small number of cones which may be red-sensitive (McCormack and Sontag 1980). However, when placed under red light, rats almost immediately show increased (night time) activity. A failure to do this indicates a serious problem (Morton D pers comm, Morton 2000).

(xv) There is evidence to indicate that rats can detect light in the ultraviolet range (Jacobs, Neitz and Deegan 1991, Jacobs, Fenwick and Williams 2001).

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Recommendations

4.2.1.1 Lighting within cages during day hours should be held at lux ranges below thresholds of aversion for rats. For most pigmented rat strains this is below 60 lux and for albino rats below 25 lux. To enable operators in rat rooms to perform visual tasks, it may be necessary to increase light levels (to approximately 210 lux at working height) for the period that the operators are in the rooms.

4.2.1.2 Light intensity can be reduced by using recessed lighting consoles in the ceiling with fluorescent lights of about 25 to 36 Watt and a low spectral intensity (wavelength) (which can be achieved by using a low colour number (for example colour 33 tubes)).

4.2.1.3 Shading should be provided over the top shelves of racks to protect rats in the top cages from overhead lights and to provide a more uniform light level between cages on different shelves.

4.2.1.4 Lighting should be diffuse and uniform to avoid glare, heat clusters and fluctuating lighting conditions for individual cages

4.2.1.5 Under bright operating lights the eyes of rats of any strain should be protected to prevent retinal damage.

4.2.1.6 Lights should be checked for flickering and any flickering rectified.

4.2.2 Light Cycles

Principles

(i) Aspects of rat physiology (for example serum triglyceride levels) show circadian rhythms (Cayen, Givner and Kraml 1972; Henning and Gisel 1980). The light/dark cycle affects the normal circadian rhythm of various parameters (such as body temperature) (Fioretti, Riccardi, Menconi et al 1973).

(ii) The light / dark cycle can also affect behaviour – for example the nocturnal feeding pattern of rats is reversed when light / dark periods are reversed (Wong and Oace 1981).

(iii) Light period protocols are not only important for the proper care of rats but they can markedly influence the outcome of scientific investigations and the interpretation of their results. For example, in a study by Dauchy et al 1997 looking at tumour growth, minimal "light leaks" of only 0.2 lux during an otherwise normal dark phase inhibited host melatonin secretion and increased the rate of tumour growth and lipid uptake and metabolism.

(iv) Setting lighting on a 12 hours light/12 hours dark cycle throughout the year effectively eliminates seasonal fluctuations. There have been few studies looking at the impact of this on biological measures. Ahlers et al 1989 showed that seasonal variations in corticosterone responses were not affected by this lighting schedule. However, changing the light/dark

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cycle has been shown to alter the pattern of immunological responses in mice (McEachron, Tumas, Blank et al 1995).

(v) The transition between light and dark can be handled by a dimmer which changes the light gradually and is preferable to a sudden turn off of light, because a sudden turn off of light gives no time for physiological and behavioural adjustment to be made. The twilight period can be quite important behaviourally (Allen 1980).

Recommendations

4.2.2.1 Regular light cycles of 12/12 – 10/14 hours light/dark are suggested. Variations in the light dark cycles to mimic seasonal changes could be considered.

4.2.2.2 The use of dimmers in rat rooms is suggested to allow the creation of twilight periods between the light and dark cycles.

4.3 Temperature

Principles

(i) Variation in temperature is one of the most obvious and important factors in a rat's environment.

(ii) The thermal biology of the rat has been extensively studied (Gordon 1990). Rats maintain metabolic homeostasis by a range of mechanisms including variation in metabolic rate, altered patterns and kinds of activities such as shivering and huddling and by creating habitats with special thermal characteristics for example, nest building.

(iii) The rat's thermo-neutral zone ranges from 27 to 30^0C (Gordon, Lee, Chen et al 1991); normothermia is maintained in ambient temperatures between 10^0C to 30^0C, rats being better able to cope with low temperatures. Vascular responses to heat and cold are seen primarily in the tail, ears and feet with approximately 20% of heat production dissipated through the tail. Fluctuations in blood flow to the feet and tail occur at ambient temperatures within the thermo-neutral zone. During heat stress, rats spread saliva over their bodies to promote evaporative cooling. Shivering normally occurs below 20^OC unless the rat is active.

(iv) Unlike other rodents, such as the mouse and the guinea pig, which select an ambient temperature associated with minimal energy expenditure, rats, when active, choose an ambient temperature which is below their thermo-neutral zone.

(v) As nocturnal animals, rats mainly rest and sleep during the day and are active at night. For the resting period, if given a choice, rats select areas with an ambient temperature of 25-30^0 C, while during the active nocturnal period they select temperatures between 17-25^0C (Clough 1982; Gordon 1990; Gordon 1993). Transition between wakefulness and sleep is sensitive to body temperature with an increase in REM sleep with increased ambient temperature; within their thermo-neutral zone, REM sleep varies significantly being

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maximal at 29^0C where it is twice that seen at 23^0C (Gao, Franken, Tobler and Borbely 1995).

(vi) In an extensive study over two generations into the effects of room temperature on reproduction, body and organ weights, food and water intake and haematological and serum biochemical measures, Yamauchi, Fugita, Obara and Ueda 1981 concluded that room temperature between 20-26^{O}C is optimum for rats, being associated with minimal variation in the measures studied. This range coincides with that of the rat's behavioural preferences (Gordon 1990).

(vii) Using measurements of tail skin temperature and behavioural responses, Yoshida and Sugiyama 1981 concluded that the optimal environmental temperature for rats is 26^{O}C irrespective of whether they were housed in a group or singly.

(viii) Variations in environmental temperature outside the compensatory capacity for rats will affect reproductive performance with decreased litter size, increased embryonic deaths and impaired growth, and cause significant variation in food and water intake and in haematological and biochemical parameters.

(ix) Gestating and lactating dams have reduced thermoregulatory ability (Knecht, Toraason and Wright 1980) and this also is true of pups where homeothermy is achieved at 3 - 4 weeks of age (Clough 1982).

(x) Temperature in the cage is influenced by cage design and construction, the position of the cage within a rack and a room, pattern of air distribution, ventilation rate, the position of the cage within the air flow pattern and its proximity to other cages (Clough and Donnelly 1984; Hirsjarvi and Valiaho 1987).

(xi) Differences between room (macro) and cage (micro) temperatures (Clough and Donnelly 1984) must be taken into account in the management of ambient temperature so as to meet the physiological and behavioural needs of the rat.

(xii) Rats generate a heat load within the cage (Besch and Woods 1977). Consequently, the ambient temperature within a cage will be determined by this heat load, the ambient room temperature, the thermoregulatory properties of the cage materials as well as the effectiveness of heat exchange by ventilation. With wire mesh cages, heat is dissipated rapidly and cage temperature and room temperature are equilibrated but rats housed in such cages have no protection from variations in room temperature and can be subjected to draughts. In cages with solid sides and floors, the cage temperature will be at least 5^{O}C above ambient room temperature but animals held in these conditions are less susceptible to fluctuations in room temperature. With solid walls and sides, heat dissipation will be influenced by the thermal properties of the cage materials, for example, stainless steel versus plastic (Hirsjarvi and Valiaho 1987). A greater disparity between cage and room temperatures can occur, depending on stocking density and the various positions of cages on a rack.

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(xiii) Under laboratory conditions, rats' abilities to control their environmental temperature have largely been replaced by external systems under human control. Where possible, strategies which enable rats to regulate or choose their microclimate, such as the provision of bedding and nesting materials and in-cage shelters, should be provided.

Recommendations

4.3.1 A room temperature range for rat housing between 20 - 26^{0}C is recommended.

4.3.2 Significant swings in room temperature should be avoided.

4.3.3 Rats should be provided with nesting materials and in-cage shelters to enable them to regulate the microclimate temperature, particularly for sleeping

4.3.4 Special attention should be given to those circumstances where the rat's thermoregulatory ability is compromised. Cage temperature for pregnant and lactating rats and pups up to 3-4 weeks of age should be at the higher end of the recommended range (24-26^{0}C).

4.3.5 If rats are held in wire bottomed cages without some solid resting area and nesting material, (for example in metabolism cages) the room temperature should be in the range of 24-26^{0}C.

4.3.6 Temperature should be monitored within the cage and at various positions within the room to monitor variation so as to optimally manage the microenvironment.

4.4 Humidity

Principles

(i) Ambient relative humidity is important to the health and well-being of laboratory rats as it influences their capacity to thermoregulate (Weihe 1965) as well as playing a role in the transmission of pathogens (Clough 1982). Environmental temperature and humidity act together on the rat's thermoregulatory ability (Weihe 1965; Clough 1982).

(ii) There is a greater risk of rats developing ringtail when they are housed at humidity levels below 40% (Flynn 1967). Low humidity (10-12%) also may contribute to the development of middle-ear disease (Lovejoy, McGuirt, Ayres et al 1994).

(iii) High humidity can enhance the proliferation of bacteria and ammonia production in cages (Reeb, Jones, Bearg et al 1998) and thus place animals at greater risk of infection.

Recommendation

4.4.1 A relative humidity at the level of rat cages of 40-70% is recommended.

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4.5 Air Quality and Ventilation

Principles

(i) Air quality is largely affected by the concentration of micro-organisms, dust particles and noxious gases, in particular ammonia and carbon dioxide. The level of exposure to these contaminants in the rat's environment can have a major impact on their health (Clough 1982; Fox 1983) and will be influenced by the relative humidity in which this occurs, the turbulence of air within the cage and the presence or absence of draughts.

(ii) Significant lung pathology has been reported in rats exposed to ammonia levels above 25 ppm, the threshold level for human safety over a 40 hour week (Broderson, Lindsey and Crawford 1976; Gamble and Clough 1976; Schoeb, Davidson and Lindsey 1982).

(iii) Volatile pollutants from soiled bedding, in concert with ammonia, produce nasal pathology in rats (Bolon, Bonnefoi, Roberts et al 1991; Ischikawa, Matsuoka and Mori 1995).

(iv) The adequacy of air exchange in the rat's immediate environment affects levels of, and variance in, environmental temperature, humidity and air quality and determines the respective levels of these measures in the macro and micro environments (Clough 1984).

(v) Air exchange is determined by the pattern of air distribution and air velocity. The placement of air inlets and outlets in a room and the rate of air exchange will affect the pattern and efficiency of air exchange. An increased ventilation rate does not necessarily mean better air exchange between cages (White 1990).

(vi) Ventilation systems in animal houses are usually set between 15 and 20 air changes per hour, although there is some debate as to the frequency required (Besch 1980; Clough 1984).

(vii) The distribution and flow of air within an animal room can play a significant role in the distribution of micro-organisms (Teelman & Weihe 1974). High ventilation rates result in greater dispersion of micro-organisms and dust but lower relative humidity and so may decrease the viability of pathogens (Clough 1984).

(viii) When housed in individually ventilated cages, rats showed a preference for conditions where air changes are kept below 80 per hour. Above this level, both heart rate and systemic blood pressure increase (Krohn, Hansen and Dragsted 2003). In this study, air speeds in individually ventilated cages of up to 0.5m/s had no demonstrable effect on the rats.

Recommendations

4.5.1 The number of air changes per hour needs to be adjusted to keep air quality, temperature and humidity at acceptable levels within cages.

4.5.2 Room ventilation rates of about 15-20 air changes per hour may be needed.

4.5.3 For rooms holding individually ventilated cages, usually 5 air changes per hour will be sufficient to maintain room air quality.

4.5.4 For individually ventilated cages, to ensure low levels of ammonia, air changes should be kept at around 50 times per hour (Krohn, Hansen and Dragsted 2003).

4.5.5 Racks should be positioned in a room so as to optimise air exchange and avoid animals being exposed to draughts.

4.5.6 Cleaning regimes should be managed to maintain ammonia levels within a cage below 25 ppm.

4.6 Sound and Vibrations

Principles

(i) There are sounds in animal rooms which may have negative effects on rats, including sounds which cannot be detected by the human ear.

(ii) Hearing in rats is acute and extends well into the ultrasonic range (Gamble 1982). In a study by Heffner et al 1994, rats were found to have a hearing range between 0.25 kHz and 70kHz. Rats are extremely sensitive to ultrasound and respond to it even under anaesthesia. The most sensitive range of hearing for rats lies largely in the ultrasonic range (20-40 kHz) (Clough 1982). Heffner et al 1994 demonstrated maximum sensitivity of hearing at 8kHz and between 32 - 38 kHz.

(iii) Vocalisations also reach into the ultrasonic range; rats use the ultrasonic range in communication and in mating (Knutson, Burgdorf and Panksepp 2002). Male rats emit a pulsed ultrasound before they copulate and an ultrasonic post-copulation call (at 22 kHz).

(iv) The same ultrasonic vocalisation (22kHz) is emitted in response to a variety of stressors and in circumstances where they are stressed, rats housed singly emit more ultrasonic calls than those housed in groups (Brudzynski and Ociepa 1992). Burman et al 2007 summarises the findings of a number of studies that indicate laboratory rats have two distinct types of ultrasonic vocalisation indicating either (mostly) positive emotional states (50kHz) or (mostly) negative emotional states (22kHz). The findings of this study indicate that ultrasonic vocalisation by rats of 22kHz could induce a negative emotional state of increased anxiety in rats hearing the vocalisation, and could therefore be a useful indicator of welfare for rat groups, including both callers and non-calling group mates.

(v) Sound can have a negative effect on behavioural patterns and physiological responses in rats (Gamble 1982) and is used as a stressor in experimental studies, although usually at

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levels above that experienced in the animal house (above 100 dB –often referred to as 'noise stress'). Differences in the magnitude and kind of physiological responses to noise stress have been found between rabbits and rats (Friedman, Byers and Brown 1967; Nayfield and Besch 1981). Examples of the effects of noise stress in rats include:

* Changes in pattern of eating behaviour (Krebs, Machr, Weyers et al 1996),
* Decreased food intake (Nayfield and Besch 1981),
* Weight loss and adrenal hyperfunction (de Boer, Slangen and van der Gugten 1988),
* Significant weight loss in pregnant rats (Kimmel, Cook and Staples 1976),
* Negative effects on cardiac responsiveness (Morvai, Szakmáry, Székely et al 1994; Breschi, Scatizzi, Martinotti et al 1994), and blood pressure (Alario, Gamallo, Villanua et al 1987),
* Exacerbation of collagen-induced arthritis (Rogers, Trentham, McCune et al 1980), and,
* Onset of autoimmune disease in neonates (Dimitrijevic, Laban, von Hoersten et al 1994),
* Changes to the morphology of intestinal mucosa (in rats exposed to 15 minutes of white noise 90dB) daily for 3 weeks (Baldwin, Primeau and Johnson 2006).

(vi) Human activity and laboratory equipment are important sources of sounds which may impact on rats (Milligan, Sales and Khirnykh 1993; Wilson and Baldwin 1998). The proximity of animal housing to construction sites can also have significant, negative effects (Fernandes and File 1993; Dallman, Akana, Bell et al 1999).

(vii) Reducing noise levels in laboratories can be difficult. If possible, equipment that emits ultrasound should not be used in an area where rats are held (for example computers and oscilloscopes). At the very least, such equipment, should be packed in screening material, such as polystyrene foam plates (which often come as packing material) to dampen the sounds (Birke 1988).

(viii) Ultrasound can be measured using equipment such as bat detectors. The monitoring and control of the acoustic environment of rats may require the input of hearing related specialists and an understanding of some of the principles of acoustics and the measurement of sound (Hughes 2007).

(ix) Exposure to music (less than 40 dB between 9.00am and 2.00pm daily) enhanced immune responses in rats (Nunez, Mana, Linarea et al 2002).

(x) Vibrations tend to have similar effects on rats as exposure to noise. Changes in the hippocampus and amygdala have been observed after rats were exposed to noise and vibration (Fernandes and File 1993). Whole body vibration (at 20Hz, 4.0g) can have similar effects as noise stress to various regions of the brain (Nakamura, Moroji, Nagase et al 1994).

(xi) To avoid vibrations, individually ventilated cages need to be checked for vibratory properties.

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Recommendations

4.6.1 Noise (loud sounds) within the human hearing range as well as in the ultrasonic range should be reduced where possible.

4.6.2 Computers, or any other equipment likely to emit high frequency ultrasonic signals, should not be used in rooms where rats are housed. If the use of such equipment is unavoidable then measures, such as packing the equipment in polystyrene foam plating, should be taken to dampen ultrasonic noises.

4.6.3 The effect of background radio sounds to alleviate the effects of ultrasound and loud noises is unclear. If a radio is used, the volume should be kept low.

4.6.4 Vibrations in rat holding rooms, and especially of cages, should be eliminated.

4.6.5 Individually ventilated cages should be checked for vibrations.

4.6.6 Due to the vibrations created, placing motorised equipment on bench tops with cages should be avoided.

4.7 Monitoring of Environmental Variables

Principles

(i) Environmental variables of the rat's living area require regular monitoring especially at the cage level. Temperature and humidity should be checked daily. Diurnal variation also should be checked where appropriate.

(ii) Temperature, humidity and air quality are affected by the system of air control.

(iii) The monitoring of ventilation in individually ventilated cages is especially important, as ventilation failure can result in death in a relatively short period .

Recommendations

4.7.1 Rat rooms should have temperature and humidity read-outs in a position where staff can easily see them.

4.7.2 Sensors should be fitted to monitor and report malfunctions in ventilation, temperature and humidity control on a 24 hour basis, with automatic alarm activation.

4.7.3 Even if centralised computer systems are used to regulate the general environmental conditions, it is still essential to check these variables regularly at the cage level.

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4.8 Cleaning

Principles

(i) There have been few systematic studies on the effects of cleaning on rat health and behaviour and not many are recent (Cisar and Jayson 1967).

(ii) Cleaning has two components: handling (see Section 3.4 Effects of Handling and Human Activity) and cleaning of cages.

(iii) Behavioural and physiological changes are seen in rats for several hours following routine cage cleaning (Saibaba, Sale, Stodulscki et al 1995; Duke, Zammit and Lawson 2001). These effects need to be taken into account when scheduling experimental procedures. In a study using male rats, Burn, Peters and Mason 2006 concluded that cage cleaning did not cause stress (according to the parameters measured) in rats (although it resulted in increased levels of play-like "skirmishing" activity). However, in breeding rats, twice weekly cleaning has been shown to cause more cannibalism of pups than weekly or every two week cleaning (Burn CC pers comm).

(iv) In a long term preference test (during dark and light periods), rats showed no preference for their own soiled bedding over clean bedding (Burn CC pers comm).

Recommendations

4.8.1 The need for changing bedding depends on the kind of bedding used and air quality. The frequency of bedding changes also will be influenced by stocking rates, strains of rats and particular disease conditions, for example, diabetes. As a guide, bedding is commonly replaced about once a week.

4.8.2 Cleaning regimes should be managed to maintain ammonia levels within a cage below 25 ppm.

4.8.3 Cleaning of cages should be done in a separate room designated for maintenance and cleaning tasks. The cage washing area should not be located near rat holding rooms to minimise disturbance from the associated activities.

4.8.4 Rat rooms should have smooth, hard and impervious surfaces throughout with no exposed joints or cracks.

4.8.5 All surfaces should be washed down periodically to keep them clean.

4.8.6 Rat holding rooms should not contain floor drains and if they do they should be rodent proof.

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4.8.7 Procedures to reduce the risk of disease spread during cleaning should be developed with particular attention to staff working in contaminated areas and with diseased animals.

4.8.8 Clean storage space for cages, food and bedding should be provided.

5. Records

5.1 Cage Labels

Recommendations

5.1.1 All cages should have labels attached to them that provide the following information, or cross reference to a central record in the same room containing this information:

- *Rat identification (strain, sex, number of rats)*
- *Age (date of birth) of litters or of individual rats.*
- *Date of entry into cage.*
- *Name and approval number of project in which rats are being used.*
- *Name, location and contact numbers of the chief investigator/teacher and, if applicable, other investigators/teachers using the rats.*
- *Name, location and contact numbers of staff associated with the housing and care of the rats.*
- *Treatments / procedures*

5.2 Breeding Records

Principles

(i) Clause 4.5.8 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states:

The person-in-charge must maintain adequate records to allow effective management of the breeding stock including the detection of the origin and spread of disease.
Records should include:
(i) the source, care, allocation, movement between locations, use and fate of all animals;
(ii) details of any diseases;
(iii) the fertility, fecundity, morbidity and mortality in breeding colonies; and
(iv) the health status, genetic constitution and physical environment of the animals.

(ii) ARRP Guideline 16: *Supervision of Animal Supply by Animal Ethics Committees* (http://www.animalethics.org.au) details the types of records that should be kept, and information that should be provided to the Animal Ethics Committee, on animal breeding activities.

Recommendations

5.2.1 To assist in monitoring the management of rat breeding colonies, regular reports must be provided to the Animal Ethics Committee, for review, on the

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fertility, fecundity, morbidity and mortality of all rat breeding colonies. The frequency of such reports should be at least 6 monthly and more often if deemed necessary by the AEC. (See ARRP Guideline 16: Supervision of Animal Supply by Animal Ethics Committees - http://www.animalethics.org.au*)*

6. Recommended Reading

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7. Additional Resources

Handle with care: An interactive training course on handling a variety of species including rats, available in video or DVD format. This is produced by the UK Institute for Animal Technology (IAT) and is an updated version of the video of the same name, with new footage. See www.iat.org.uk

Pain Assessment in the Rat: A CD-rom produced by the Comparative Biology Centre, University of Newcastle-Upon-Tyne. This provides guidance for assessing rats according to the scheme developed by Dr Roughan and Professor Flecknell. See www.lal.org.uk/digital.htm

The Laboratory Rat: A Natural History:: This is a training video that follows a group of laboratory rats which has been released into a large outdoor enclosure. It provides an excellent illustration of the important behaviours described in section 1.5. More information is available at www.ratlife.org

The rat – recommended technical procedures: This is a training video (24 minutes) produced by the Canadian Association for Laboratory Animal Science as an introduction to safe handling techniques and methods for injection and blood sampling in the laboratory rat. Purchasing details: http://www.calas-acsal.org

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