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How to improve housing conditions of laboratory animals: The possibilities of environmental refinement

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FOREWORD

New Zealand's *Good Practice Guide for the use of animals in research, testing and teaching* states that wherever possible, research animals should be provided with "an environment that can accommodate the behavioural and physiological needs of the species".

Traditionally, laboratory animal housing has been based on economic and ergonomic considerations, but growing awareness of animal welfare has brought an increased focus on providing less barren environments for these animals. In seeing environmental refinement as an important consideration under the "refinement" aspect of the Three Rs, the National Animal Ethics Advisory Committee (NAEAC) has selected this paper for publication as the tenth in its Occasional Paper series as a comprehensive and readable review of what we see as a complex but important issue.

We would encourage all AEC members, researchers and animal care staff to read this excellent paper by Vera Baumans and Pascalle Van Loo, which is reprinted with permission from *The Veterinary Journal.*

Virginia Williams
Chair, NAEAC
January 2014

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How to improve housing conditions of laboratory animals: The possibilities of environmental refinement

V Baumans and P L P Van Loo

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Abstract

Housing systems for captive animals have often been designed on the basis of economic and ergonomic considerations, such as equipment, costs, space, workload, ability to observe the animals and to maintain a certain degree of hygiene, with little or no consideration for animal welfare. Environmental refinement can be defined as any modification in the environment of captive animals that seeks to enhance the physical and psychological well-being of the animals by providing stimuli which meet the animals' species-specific needs. This article provides an overview of environmental factors that influence the well-being of captive animals with specific reference to the needs of the most common laboratory species.

It is important to evaluate environmental refinement in terms of the benefit to the animal, by assessing the use of and preference for certain enrichment, the effect on behaviour, and the performance of species-typical behaviour on physiological parameters. It is also necessary to evaluate the impact of refinement on scientific outcome, including whether and how statistical power is affected. Communication and team work between animal welfare scientists, animal research scientists, institutional animal welfare officers, veterinarians and animal ethics committees, animal facility management and personnel, are essential for success.

Introduction

The use of animals in experimental research parallels the development of medicine, which had its roots in ancient Greece. Today, 75–100 million vertebrates are used annually worldwide in research and testing for a wide range of purposes. Mice and rats are the most frequently used animal species (Baumans, 2010a).

The increasing demand for high standard animal models and widespread criticism of the use of animals led to the development of Laboratory Animal Science in the 1950s, a field that can be defined as a multidisciplinary branch of science, contributing to the quality of animal experiments and to the welfare of laboratory animals. Guiding principles are the three Rs of replacement, reduction and refinement, introduced by Russell and Burch in 1959 in their book *The Principles of Humane Experimental Technique.*

Replacement means substituting live animals with in vitro techniques (e.g. cells, tissues), or the use of lower organisms e.g. yeasts, computerised models, genomics, etc. Reduction means decreasing the number of animals used by calculating more accurately the number of animals per experimental group, using applying statistics prior to the experiment, e.g. power analysis. Reduction also encompasses standardisation of animals in terms of genotype and microbiological quality, experimental procedures and the environment, including standardised food and climate in the animal room. Refinement means decreasing discomfort by providing adequate anaesthesia, analgesia and care, and by guaranteeing the skills of the researcher/animal staff. This can be achieved by education and training, by improving experimental procedures and by determining a humane endpoint, where the animal is euthanased to prevent unnecessary suffering. With regard to housing conditions, refinement can be achieved by translating the behavioural and physiological needs of the animal into adequate housing and husbandry. Although most emphasis has been placed on mice and rats in this review, some other species also used as pet animals are included for the interest of the reader.

Housing conditions and its effect on animals

Housing systems for captive animals have often been designed on the basis of economic and ergonomic concerns such as equipment, costs, space, work load, and the ability to observe the animals and to maintain a certain degree of hygiene, with little or no consideration for animal welfare. Standardisation of the animal cage was considered essential to reduce variation, resulting in a shoebox-shaped cage and standard bedding material for rodents.

However, over the last decade there has been recognition of the importance of providing an environment which meets species specific needs, including environmental complexity and social housing, in order to improve their wellbeing (Olsson and Dahlborn, 2002; Baumans, 2005; Hubrecht, 2010; European Directive 2010/63/EU, 2010; NRC, 2011).

The environment of an animal consists of a wide range of stimuli, including the social environment (animals from the same or from other species, including humans) and the physical environment, such as the animal room (e.g. climate, illumination, noise, vibration), and the cage and its contents (Lutz and Novak, 2005; Baumans et al., 2010b; NRC, 2011; Fig. 1). Environmental conditions such as housing and husbandry have a major impact on the laboratory animal throughout its life, not only during the experiment, but also before and after the study period (Eskola et al., 1999; Würbel, 2001; Baumans, 2005, 2010d; Hubrecht, 2010).

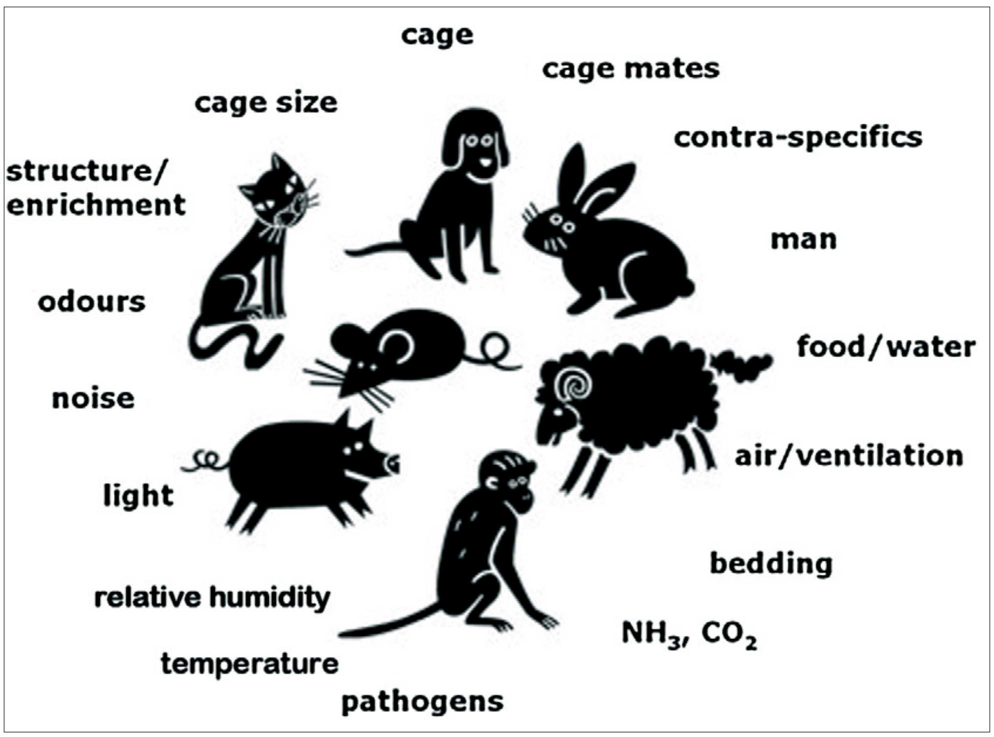

Figure 1: The environment of the laboratory animal.

The traditional care and maintenance of laboratory animals does not usually consider the species-specific needs in relation to housing and feeding regimens. For instance, most of the wild counterparts of laboratory animal species forage in their habitat, which occupies a great deal of their time. They also might hoard their food, as hamsters do. In the laboratory, animals are provided with readily available food from the food hopper, resulting in considerable unoccupied time, which could cause boredom and consequently might lead to abnormal behaviour, such as stereotypic behaviour (bar gnawing, hair pulling, circling, etc.; Baumans, 2005). The variability in specific needs not only differs between species, but also varies among strains within a species, due to variability in the genetic background (Van de Weerd et al., 2002; Olsson and Dahlborn, 2002; Hubrecht, 2010).

Laboratory animals have partially adapted to captive life, but still show similarities to their wild counterparts (Berdoy, 2002; Baumans, 2005). The animal's environment should cater for innate physiological and behavioural needs, such as social contact, nest building, hiding, exploration, foraging, gnawing and resting. Most laboratory animal species, such as rodents and rabbits (but also goats, sheep and pigs) are highly susceptible to predators. Therefore, they are likely to show strong fear responses in unfamiliar circumstances if they cannot shelter, resulting in attempts to flee, biting when handled or sudden immobility to avoid being detected (Hurst, 1999; Ladewig, 2000; Hubrecht, 2010; Holtz, 2010). For this reason, cages and pens should include hiding places. Ideally, the animal should feel secure in a complex, challenging environment, which it can control (Poole, 1998). Security can be achieved by providing nesting material, hiding places and compatible cage mates (Olsson and Dahlborn, 2002; Hubrecht, 2010). Even simple environmental refinement induces a robust and repeatable anxiolytic effect in mice (Sztainberg and Chen, 2010).

However, laboratory animals are usually housed in relatively barren cages and provided with ad libitum food, which frequently causes adverse effects on the animal's behaviour and physiology, resulting in a shortened life span due to overfeeding and inactivity (Mattson et al., 2001). Standardisation of environmental conditions serves to reduce individual differences within animal groups (intra-experiment variation), ultimately facilitating the detection of treatment effects. It also reduces differences between studies (inter- experiment variation), ultimately increasing the reproducibility of results across laboratories (Olsson et al., 2003).

Nevertheless, it has been shown that despite rigorous efforts to equalise conditions across sites, different inbred mouse strains, which originated simultaneously from three well-recommended laboratories, demonstrated significant effects associated with their respective sites for nearly all variables examined (Crabbe et al., 1999; Wahlsten et al., 2003). Barren, restrictive and socially-deprived housing conditions interfere with the development and function of brain function and behaviour in rodents (Benefiel and Greenough, 1998; Renner and Rosenzweig, 1987; Würbel, 2001) and can cause abnormal behaviour, such as stereotypic behaviour and barbering (incessant grooming), which can be counteracted by a more complex environment (Bechard et al., 2011; Gross et al., 2011). Restrictions in housing conditions for pigs which prevent them from rooting are potentially stressful, evidenced by increased cortisol responses (Ladewig, 2000). Ironically, the barren environment that has been designed to minimise the effects of an uncontrolled environment on laboratory animals might be a primary source of pathological artefacts.

Appropriate structuring of the cage/pen environment is typically more beneficial than the provision of a larger floor area; however, a minimum floor area is necessary to provide a structured space. Except for locomotor activity (e.g. playing), animals do not actually require much space but instead, they need resources and structures within an area for specific behaviours (Olsson and Dahlborn, 2002; FELASA, 2006; European Directive 2010/63/EU, 2010; Baumans, 2010d; NRC, 2011).

It is difficult objectively to specify minimum cage sizes for maintaining laboratory animals, as much depends on their strain, group size and age, their familiarity with each other and their reproductive condition. Cage sizes recommended in current European guidelines on accommodation for laboratory animals are preferably based on scientific evidence, but where this is lacking or insufficient, recommendations are also based on best practice.

Appendix A of the Council of Europe (2009) in the Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS123), the Annex III of the European Directive 2010/63/EU (2010) and the *Guide for the Care and Use of Laboratory Animals,* (NRC, 2011), provide guidelines for accommodation and care of animals. It is stated in the European Directive that '*All animals should be allowed adequate space to express a wide behavioural repertoire. Animals should be socially housed wherever possible and provided with an adequately complex environment within the animal enclosure to enable them to carry out a range of normal behaviours*'. The *Guide for the Care and Use of Laboratory Animals* (NRC, 2011) even states that '*At a minimum, animals must have enough space to express their natural postures and postural adjustments without touching the enclosure walls or ceiling. . .*'

Environmental enrichment/refinement

One of the possibilities for improving living conditions of laboratory animals is to provide opportunities for the animals to perform a more species-specific behavioural repertoire by providing environmental enrichment. Environmental enrichment can be defined as any modification in the environment of captive animals that seeks to enhance its physical and psychological well-being by providing stimuli which meet the animals' species-specific needs (Newberry, 1995; Baumans, 2005). This has been introduced increasingly into laboratory animal research facilities (Olsson and Dahlborn, 2002).

In neuroscience research the term 'environmental enrichment' is frequently used, although this type of environmental enrichment is based mainly on novelty-induced stimulation and regularly changing items, primarily to measure the effects on brain neuronal plasticity (Mohammed et al., 2002). Neuroscience studies of enrichment often use the term 'impoverished' to signify conditions of individually housed subjects in conventional cages (Mohammed et al., 2002), whereas 'standard conditions' refers to socially-housed subjects in conventional cages. 'Enriched conditions' describes larger cages with many different kinds of enrichment objects and a larger number of animals. This is in contrast to the enhancement of animal welfare by means of appropriate enrichment focused on animal needs (Baumans, 2005). The term 'enrichment' implies a luxury of some sort, while the term 'needs' on the other hand, implies a necessity. For that reason, it is better to use the term 'environmental refinement', when applied to laboratory animals, instead of 'environmental enrichment'. Environmental refinement is an ongoing process and we should aim to provide stimuli beyond the satisfaction of basic needs normally catered for in standard housing conditions. From a welfare point of view, this seems to be a positive development as it is generally accepted that the provision of environmental refinement improves the animal's well-being.

Refinement of the animal's environment can be focused on both the social environment (social partners, including human beings), and the physical environment, consisting of sensory stimuli (auditory, visual, olfactory and tactile) and nutritional aspects (supply and type of food). Furthermore, psychological appraisal of the environment includes concepts such as controllability and predictability (Van de Weerd et al., 2002) and cognitive performance, which can be enhanced by structuring the cage with nest boxes, tubes, partitions and nesting material (Manser et al., 1998; Latham and Mason, 2004; Sherwin and Nicol, 1997; Ras et al., 2002; Baumans, 2005; Hutchinson et al., 2005; Jeong et al., 2011). Van de Weerd et al. (1997) showed that nesting material such as tissue paper was highly preferred by mice (Fig. 2). A recent study showed that lack of nesting material produced substantial increase in anxious behaviour and that learning and memory was negatively affected (Kulesskaya et al., 2011).

For gregarious species, social contact is a stimulating part of their environments and should only be denied in exceptional cases e.g. extreme aggression in males from certain mouse strains, rabbits, dogs and non-human primates (Van Loo et al., 2003; Lutz and Novak, 2005; Wersinger and Martin, 2009; European Directive 2010/63/EU, 2010; Hubrecht, 2010; NRC, 2011). Social interactions are important contributors to animal welfare, provided that the group composition is appropriate for the species. Group-housed animals are able to engage in social exploration, but the behavioural activities of one animal, such as scent marking or digging, can also be a valuable source of novelty that elicits exploration by other individuals (Olsson and Westlund, 2007).

Social housing is beneficial only if the pairs or groups are harmonious and stable (European Directive 2010/63/EU, 2010; NRC, 2011). The successful establishment of groups requires the grouping of individuals that are compatible, a characteristic that is strongly influenced by age, sex, and hierarchical rank; some of these can be managed by good husbandry practices. The establishment of groups or compatible pairs must be handled with care, particularly in the case of male mice and rabbits, adult hamsters or gerbils, mini pigs and non-human primates, which can exhibit severe conspecific aggression. The provision of visual barriers and refuges that allow animals to withdraw out of sight when a threat occurs can reduce aggression.

Additionally, the disruption of established stable and harmonious groups can be very stressful and should be minimised (Van Loo et al., 2003). One option for preventing aggression in male rabbits is castration (Lidfors and Edstroem, 2010), when it does not interfere with the experimental design. An aggressive male mouse could be housed together with an ovariectomised female as a companion when the animals have to be kept for long periods (Wersinger and Martin, 2009), although it has potential for causing significant distress in the ovariectomised females paired with aggressive males, depending on the mouse strain. Moreover, consideration needs to be given to whether the negative impact of the surgical procedure is outweighed by the benefit of social housing. The effect of the different smell of neutered animals on intact animals is not known.

Figure 2: Paper nesting material as cage refinement for laboratory mice.

Van Loo et al. (2007) assessed several physiological and behavioural parameters in mice after surgery for implantation of a telemetry transmitter and found that, with respect to postoperative recovery and distress, they benefited most from being socially housed. However, when social housing is not possible, individual caging appears to be a better option than separating mice by a grid partition, possibly because they are frustrated by not being able to huddle together.

Species specific needs and their integration in housing systems

Environmental refinement should meet the animal's needs, be practical, inexpensive and pose no risk to humans, animals, or to the experiment (Baumans, 2005). Refinement items should be designed and evaluated, based mainly on knowledge gained in refinement studies (Van Loo et al., 2005).

Rodents

Nesting material is important for rats, mice, hamsters and gerbils as it enables them to create an appropriate, secure microenvironment for resting, hiding, huddling and breeding (Manser et al., 1998; Ras et al., 2002; Latham and Mason, 2004; Sherwin and Nicol, 1997; Jeong et al., 2011). It has been suggested that a constant background noise, such as a radio, has some benefits in facilitating breeding and making animals less excited, although it could also be a stressor (Baumans, 2010d). Krohn et al. (2011) showed that rats react to different sound patterns from a radio, but also that the reaction might vary by rat strain.

In the 1960s, wire bottom cages were used for the convenience of husbandry practices such as cleaning, often leading to foot injuries in laboratory animals (NRC, 2011). This led to the introduction of solid bottom cages in the 1990s, which were usually only equipped with bedding, food and water (Baumans et al., 2010c). Nowadays, alternative housing systems that cater for the behavioural needs of rats and mice are available commercially e.g. elevated cage tops and bi-level cages that allow exploration such as climbing and rearing, and provide shelter. Figs. 3a–c provide a historical overview of what has been considered standardised housing for laboratory rats.

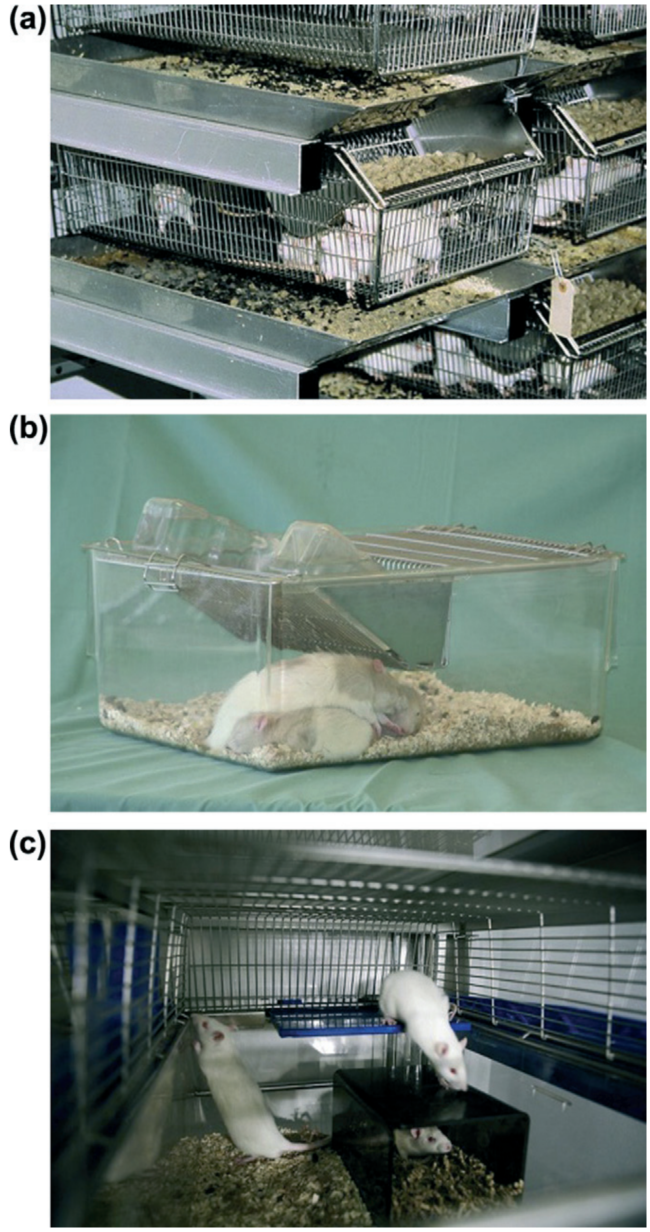

Figure 3: Standard housing for rats over the course of 50 years. (a) Standard housing for rats around 1960; (b) Standard housing for rats around 1990; (c) Standard housing for rats around 2010.

A recently introduced source of potential discomfort could be individually ventilated cage systems (IVCs). The use of IVC systems started 30 years ago and they are now frequently used, especially for housing transgenic or immune-compromised rodents. Typically, each cage can be ventilated with 25–120 air changes/h with the air blown into the cage at a relatively high speed. The advantages of the system are the improved protection of the animals against microorganisms at cage level and of the animal staff against allergens, the improved microclimate and the reduced need for cage cleaning (Hoglund and Renstroem, 2001; Renstroem et al., 2001). However, health monitoring and inspection of the animals can be difficult, and procedures and cage cleaning can be more time-consuming due to the requirement that procedures must be performed in laminar air flow chambers to maintain the advantages of IVCs (Renstroem et al., 2001). Moreover, the high intra cage ventilation rate could induce chronic stress and heat loss by draughts (Baumans et al., 2002; Krohn, 2002).

Humans consider air speeds <0.2 m/s to be a draught and this is also generally agreed to be an upper limit for rodents (Lipman, 1999). Preference tests have shown that rats choose cages with >80 air changes/h (Krohn et al., 2003). The physiology and behaviour of mice were not adversely affected by <80 air changes/h when an air inlet was located at the top of the cage and nesting material was provided (Baumans et al., 2002). This means that the location of the air supply to the cage (from the wall or from the top), the ventilation rate and the presence of nesting material are all important considerations when assessing the impact of IVC housing on the well-being of mice. Evidence that animals are reacting to draughts could be a change of location of the nest and the building barriers of bedding.

Mice

Van de Weerd et al. (1997) showed that nesting material such as tissue paper was highly preferred by mice (Fig. 2). Lack of nesting material produced substantial increase in anxious behaviour and affected learning and memory negatively (Kulesskaya et al., 2011). Reduction in nest building is also used as an indicator of compromised welfare (Hawkins et al., 2011). Besides nesting, mice also benefit when their exploratory, foraging and social needs are met by providing them with objects, such as cardboard, plastic tubes, gnawing sticks, opportunities to be engaged in finding food and contacts with social partners (Hutchinson et al., 2005; Baumans, 2010d).

Rats

Like mice, rats are a prey species and this is clearly shown in their behaviour. Rats are social nocturnal burrowing animals, hiding during daytime and fleeing easily. It was shown that in a semi-natural environment, laboratory rats, bred for generations in captivity, started immediately to make burrows and nests (Berdoy, 2002). Thus, nest building is common in rats when nesting material is available. However, when paper nesting material is provided for the first time to adult rats, they will generally chew and eat it. In contrast to mice, rats need to learn to make nests from their mothers (Van Loo and Baumans, 2004). Rats benefit from nesting material, nest boxes or other refuges and chewable toys and they prefer chewable over non-chewable objects (Manser et al., 1998; Ras et al., 2002; Hutchinson et al., 2005). Rats also show a clear preference for dark nest boxes (Manser et al., 1998). Their needs for foraging and social contact are comparable with mice (Hutchinson et al., 2005).

Guinea pigs

Guinea pigs are cursorial rodents which do not burrow, but which can live in burrows made by other animals in the wild. Refuges such as tubes or shelters should be provided in the cage or open to allow guinea pigs to climb onto or hide under them, as they are easily frightened. Hay will satisfy the need for roughage and shelter and wood sticks can be used for chewing and gnawing (FELASA, 2006; Kaiser et al., 2010). As guinea pigs are social animals, they should be housed with social partners e.g. in pairs, in harems, or in female groups. Males are preferably housed in pairs (Kaiser et al., 2010).

Gerbils

Gerbils enjoy digging and in the wild they build extensive tunnel systems. Laboratory-housed gerbils often develop stereotypic digging behaviour unless they are provided with adequate facilities (Waiblinger, 2010). For this reason, gerbils need comparatively more space so that they can build or use burrows of sufficient size. They also require a thick layer of litter for digging and nesting in and/or a burrow substitute, which might need to be up to 20 cm long. Nesting material (hay, straw, etc.) and wood sticks can be used for chewing and gnawing (FELASA, 2006; Waiblinger, 2010). Gerbils can be housed in opposite-sex breeding pairs or in same-sex groups of two or more animals. Regrouping should be avoided because of potential aggression (Waiblinger, 2010).

Hamsters

Hamsters are burrow-digging, nest building rodents (Whittaker, 2010). The wild ancestors of the hamster were largely solitary except for mating. Group housing is possible, but special care should be taken in forming socially harmonious groups, as they sleep and huddle together. Aggressive animals, especially females, should, in our experience, be separated. Minimum environmental refinement should include nesting material, a refuge area (e.g. tube, hut), roughage and gnawing objects (FELASA, 2006; Baumans et al., 2010b; Whittaker, 2010).

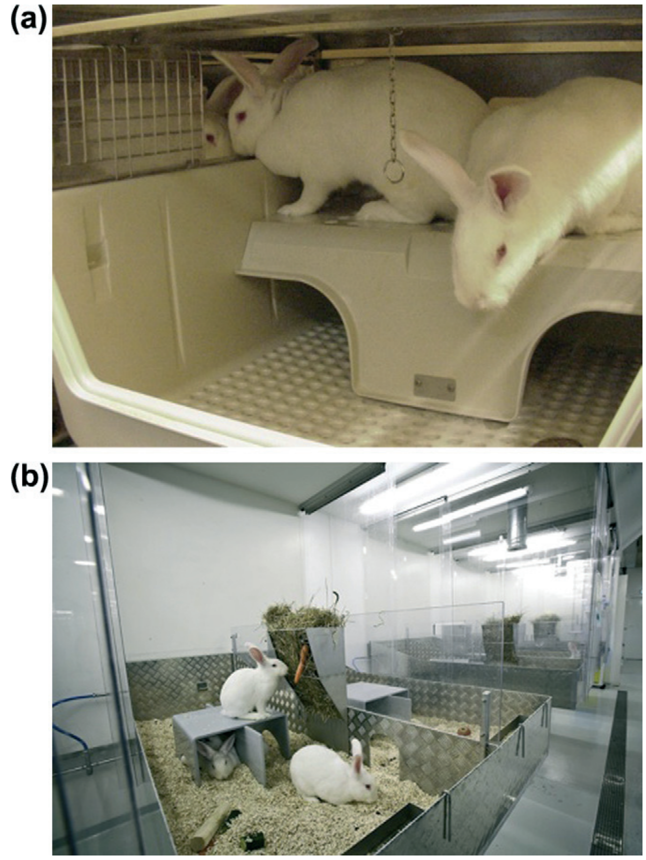

Figure 4: Example of standard housing for rabbits in two different animal facilities in Europe. (a) Standard rabbit housing at GDL, The Netherlands; (b) Standard rabbit housing at Novo Nordisk A/S, Denmark.

Rabbits

Traditionally, it has been common practice to keep rabbits individually in cages, although many facilities are now replacing this approach with pen housing. Housing rabbits in small cages limits their freedom of movement and prevents normal behaviour, such as hopping and rearing. This lack of normal movement could lead to the development of pathological changes such as osteoporosis (Morton, 1993). One often-used solution is to connect two cages together so that rabbits can be pair-housed, or at least move between cages. Pen housing might be an even better option and is widely used for female rabbits and also for castrated males (Figs. 4a and b). The physical environment for rabbits can be improved by providing roughage, hay blocks or chew sticks, as well as a raised area for withdrawal and a lookout such as a platform (FELASA, 2006; Council of Europ, 2009; European Directive 2010/ 63/EU, 2010; Lidfors and Edstroem, 2010).

In the wild, rabbits control their environment by sitting in an upright position, which is mimicked by the platform (Stauffacher, 1995; Hubrecht, 2002; Baumans, 2010a). Nesting material and a nest box or other refuge should be provided for breeding does. In floor pens used for group housing, visual barriers such as physical structures could meet some of the species-specific needs of these animals (Ottesen et al., 2004; Lidfors and Edstroem, 2010).

Dogs

Dogs should be held in socially harmonious groups or in pairs (FELASA, 2006; Council of Europ, 2009; European Directive 2010/63/EU, 2010). Care should be taken to ensure that low-ranking dogs are not deprived of resources, such as food or access to a resting area (Hubrecht, 2010). A recent study reported that housing with a familiar conspecific resulted in fewer vocalisations (Hubrecht, 2010).

As chewing is an important behaviour, items such as long-lasting chew toys or synthetic hides should be provided to meet this need. Dogs will make extensive use of these items, particularly if they are flavoured. Proper presentation, for example by suspending chews a few centimetres from the floor of the pen on a spring, can help to minimise cleaning and possessive aggression problems, while also allowing the animals to chew in a species-specific manner. Dogs make extensive use of platforms for

Figure 5: Platforms and toys as cage refinement for laboratory dogs.

play and rest and for observation of events outside their enclosures (Hubrecht, 2002; Ottesen et al., 2004). As with rabbits, a higher position in the enclosure such as a platform or shelf helps to meet the dog's need for control of the environment (Fig. 5).

Cats

Cats originate from a largely solitary-living species. They avoid physical confrontation by using behaviours to maintain distance, such as olfactory marking, posturing and vocalisation. They try to evade threats from other cats by hiding or fleeing to elevated locations (McCune, 2010). However, they can be housed in compatible groups or in pairs. The enclosure should include shelves at different heights and a choice of comfortable resting and hiding places. A significant reduction in stress was noted when cats were provided with a cardboard hiding box compared to control cats housed only with an open resting place (McCune, 2010).

Mini pigs

Pigs and mini pigs are sociable and exploratory animals (Holtz, 2010). Whenever possible, pigs and mini pigs should be purchased in groups already established from familiar or socially compatible animals. A social dominance order is quickly established. As males in the wild tend to be solitary, except during the mating season, sexually mature males might fight fiercely when housed together (Holtz, 2010). They should have permanent access to sufficient straw, hay or sawdust to enable proper investigation and manipulation activities such as rooting. Hay and straw are the materials of choice for mini pigs, as they prevent gastric mucosal hyperkeratosis and provide environmental refinement. Balls with a hole where food pellets can be hidden can be used for play and foraging (FELASA, 2006). When given the opportunity, mini pigs are clean, choosing specific sites for defecation and urination, while keeping their nest area clean (Holtz, 2010).

Non-human primates

Primates are highly social animals. Being part of a compatible group such as a kin-bonded group consisting of close relatives provides a sense of security for the vast majority of non-human primates

(JWGR, 2009; Buchanan-Smith, 2010). It also provides opportunities for a whole range of species-specific social activities, such as grooming, embracing, huddling, patting and kissing. Singly housed primates are particularly prone to abnormal behaviour, but keeping them in groups reduces the prevalence of this behaviour (FELASA, 2006; Buchanan-Smith, 2010; Wolfensohn, 2010).

Enclosures for primates should enable them to fully utilise the vertical dimension and active forms of environmental refinement should be provided e.g. toys, swings, perches, branches, ladders, chains, and/or car tyres, which allow them to carry out a normal behavioural repertoire (Wolfensohn, 2010). However, toys might lose their novelty quickly and should be changed frequently in a rotational scheme (Lutz and Novak, 2005; Wolfensohn, 2010). Nest boxes should be provided for marmosets and tamarins as sleeping sites, as they provide comfort and security (Buchanan-Smith, 2010).

Non-human primates in the wild spend a considerable amount of time searching and foraging for food, whereas in captivity they are provided with readily available food, leading to unoccupied time, which might trigger abnormal behaviour (Lutz and Novak, 2005). Fruits, nuts, whole coconuts with intact shells, frozen grapes, puzzle feeders and foraging devices containing a mixture with sawdust, dried fruits or mealworms are effective in reducing stereotypic behaviour and increasing activity (Reinhardt, 2002, 2010; Lutz and Novak, 2005; Buchanan-Smith, 2010; Wolfensohn, 2010).

Passive forms of refinement include television, videotapes, mirrors and auditory stimulation including music, or sounds from nature. Mirrors can be useful so that primates can view their reflection as a conspecific or to allow them to view activities outside the enclosure (Lutz and Novak, 2005; Reinhardt, 2010). Refinement should be tailored to the species, gender and age of primate, and ideally to the individual animal. The most effective form of refinement for captive primates is social housing, which seems to be the most effective way to prevent the development of abnormal behaviour in young primates (Lutz and Novak, 2005).

Considerations in the implementation of environmental refinement

There is concern in the scientific community regarding the possibility that environmental refinement conflicts with the standardisation of experiments. Standardisation increases the reproducibility and comparability of experiments. It aims to reduce unwanted variation caused by animal and environmental factors, thereby minimising the number of animals required. Some researchers are of the opinion that animals from a refined environment show more variability in their response to experimental procedures because they show more diverse behaviour.

In complex environments, animals are not just responding to one stimulus in isolation, but to many variable stimuli at once, causing increased variation (Eskola et al., 1999; Tsai et al., 2003). However, it could be argued that because animals can perform more species-specific behaviour in refined environments, they might be better able to cope with unexpected events and thus show a more uniform response. Animals from refined housing conditions are expected to be physiologically and psychologically more stable and they might therefore be considered as superior animal models, ensuring more generalisable results (Poole, 1997). Several studies have shown that the provision of environmental refinement can lead to more variable results, hence increasing the number of animals necessary to achieve sufficient statistical power, whereas others have shown that variability is unaffected or even decreased (Eskola et al., 1999; Mering et al., 2001; Van de Weerd et al., 2002; Olsson and Dahlborn, 2002; Augustsson et al., 2003; Tsai et al., 2003; Mikkelsen et al., 2010; Shair et al., 2011). Simpson and Kelly (2011) conducted a literature search and reported that the most important variables to consider in the implementation of environmental refinement were cage size and housing density; age, sex and strain; duration of environmental refinement; the environmental refinement protocol and items employed; and the use of appropriate controls.

Interestingly, despite an increase in variation, statistical power could increase due to larger differences in means (FELASA, 2006; Würbel, 2007). Moreover, if environmental refinement increases experimental animal variation, it is important to determine whether a slight increase in variation is biologically relevant or not.

It has been argued that systematic variation improves the reproducibility of animal experiments (Richter et al., 2010). Würbel and Garner (2007) state that standardisation is a flawed concept which risks obtaining results of poor external validity and therefore needs to be completely revised. Whether the outcome of these studies is positive or negative with regard to provision of environmental refinement heavily depends on the type of refinement provided, the parameters measured, the age and gender of the experimental animals and the interpretation of the results (Van de Weerd et al., 2002; Baumans, 2005).

Environmental refinement should comprise a well-designed and critically evaluated program that benefits the animals as well as the experimental outcome. It should not be a process of randomly applying objects that staff consider attractive for the animals without proper evaluation (Van Loo et al., 2005). To implement environmental refinement successfully in a laboratory setting, it is crucial that all factors important to the animal, the scientific validity of the animal model, and the animal facility are equally addressed. For the animal, it is most important that the environmental refinement program benefits its welfare. For the scientific validity of results, it is most important that the environmental refinement program leaves the animal model intact, and that even if results differ from historical data, valid conclusions can still be drawn. For the animal facility, the most important factors are the institution's internal work culture, costs and work load vs. work satisfaction. However, when addressed properly prior to implementation, an increase in workload is usually not considered a problem, since animal care takers in general report a higher level of job satisfaction due to the obvious benefits for the animals and a strengthened human–animal bond (Baumans et al., 2010c).

Environmental refinement should be regarded both as an essential component of the overall animal care program and as important as nutrition and veterinary care. The key components of a refinement program are the animal staff, who must be motivated and educated (Stewart and Bayne, 2004; NRC, 2011) and the contribution of the research community. It is critically important to evaluate the animal benefits of environmental refinement by assessing the species-specific preference for certain enrichment, its effect on behaviour and the performance of species-typical behaviour, and any changes to physiological parameters (Ottesen et al., 2004; Baumans et al., 2010c). It is also necessary to evaluate the impact of environmental refinement on scientific method, outcome and whether and how statistical power is affected (Baumans et al., 2010c; Pasalic et al., 2011; Shair et al., 2011). Communication and team work between animal welfare scientists, animal research scientists, institutional animal welfare officers, veterinarians and animal ethics committees, and animal facility management and personnel is essential for successful environmental refinement (Ottesen et al., 2004; Weed and Raber, 2005; FELASA, 2006; Lloyd et al., 2008).

Conclusions

Successful implementation of environmental refinement is reached by optimising the balance between scientific validity, animal welfare and job satisfaction for animal care staff. The institution as a whole plays a key role in providing the means and opportunities needed, which will, in turn, contribute to overall corporate quality and corporate social responsibility.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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