A REVIEW OF THE HOUSING CONDITIONS FOR LABORATORY ANIMALS

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Summary

The need for laboratory animals for the use of research is generally acknowledged by society. However, the use of animals for biomedical research has been a topic of debate for many years and raises ethical and moral concerns. An increasing demand for high standard animal models has led to guidelines and research focusing on or taking into account laboratory animal welfare and the quality of animal research and hereby also the development of the three R’s: Replacement, Reduction and Refinement. The three R’s are used in many countries and are incorporated into legislation and guidelines. This master thesis will focus on Refinement and provides an overview of the housing conditions of several countries/continents for the most commonly used laboratory animals (e.g. mice, rats, guinea pigs and non-human primates). By using legal documents of Europe, The United States, United Kindom, Australia and Canada as well as scientific research, well balanced standards and objective criteria regarding housing conditions and housing environment for laboratory animals are formed. Criteria concerning the micro-environment are given which includes cleaning routines, cage and space requirements, floors, bedding and nesting material, enrichment and social housing. Requirements for the macro-environment include temperature, ventilation, illumination, noise and humidity. Throughout this thesis it should become clear that each component of the micro- and macro-environment are very important to the health and welfare of the animals and therefore can have a large influence on the quality of life. Furthermore, each component should be carefully chosen/designed with consideration of the other components (e.g. the choice of ventilation will influence the humidity levels or different cage designs will require different ventilation rates). Extra measures on how to improve the welfare of laboratory or captive non-human primates are deliberated in the discussion due to the high cognitive capacities and sentiency of certain primate species which makes it difficult to house them.

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1. Introduction

Since the 70s, the total number of biomedical articles published annually more than doubled. However, the proportion of studies using animals has decreased by thirty percent and the average number of animals used per published paper has decreased by fifty percent (Carlsson et al. 2004). Still, up to this day 75 – 100 million vertebrates are used annually worldwide for research and testing purposes where mice and rats are used most frequently (Baumans et al. 2010a; 2013). A more recent report from the commission to the Council and the European Parliament also found a decrease in the number of animals used for research from 2008 to 2011 (i.e. a reduction of roughly half a million animals) (European Commission 2013). The need for animals in biomedical research is acknowledged but also brings ethical concerns and moral issues which have been debated over the years. The increasing demand for high standard animal models has led to the development and increased implementation of the three R's: Refinement, Replacement and Reduction (Russel and Burch 1959). The three R's are used in many countries as a basis for legislation and guidelines for laboratory animal research (Carlsson et al. 2004). Implementation of the three R's can contribute to the quality of animal research as well as the well-being of the animals (Bauman et al. 2013). Reduction means reducing the number of animals used for the research by means of using statistical programs prior to the experiment in order to predict the number of animals needed to gain a significant outcome. Replacement means replacing live animals with either use of lower organisms (e.g. yeasts, Drosophila flies or Caenorhabditis elegans worms), computational models, genomics or replacing the animal by *in vitro* techniques (e.g. cells or tissues) (Baumans et al. 2013). Refinement means reducing the discomfort of the animal by means of providing the right anesthesia, analgesia, humane endpoints (i.e. decision to end suffering by euthanasia) and care by properly trained researchers and staff or improving welfare of animals. Moreover, refinement may also pertain to housing conditions. Housing systems have often been designed on the basis of ergonomic and economic concerns without regards of the behavioral, physical and psychological needs of the animal used (Baumans et al. 2013).

This thesis will be focused on refinement and particularly on laboratory animal housing. An overview of laboratory husbandry specifications from several countries/continents, based on documents and literature, as well as balanced standards that can be used for laboratory animal facility design in emerging economies will be provided. The aim is to form objective criteria regarding housing conditions and housing environment of the most common animal species used in these emerging countries: rats (derived from *Rattus norvegicus)*, mice (derived from *Mus musculus)*, guinea pigs (derived from *Cavia porcellus)* and non-human primates (NHPs) such as marmosets (*Callithrix spp.),* tamarins *(Saguinus spp.)* squirrel monkeys *(Saimiri spp.),* macaques (*Macaca* spp.), vervets (*Cercopithecus aethiops* or *Chlorocebus aethiops),* baboons *(Papio*, *Theropithecus* and *Mandrillus)* and chimpanzees *(Pan troglodytes).* In order to create specific housing requirements, guidelines from different countries have been used as the primary resource next to scientific research articles:

- *The United States*: The Guide for the Care and Use of Laboratory Animals. Institute for Laboratory Animal Research, National Research Council [NRC] 2011.
- *Canada*: Canadian Council on Animal Care [CCAC]: Guide to the Care and Use of Experimental Animals, volume 2; 1984 and Laboratory Animal Facilities-Characteristics, Design and Developments 2003.
- *Europe*: The Convention for the Protection of Laboratory Animals of the Council of Europe, ETS no.123, Appendix A (2006) and Council Directive 2010/63/EU of the European Commission, Annex III (2010).
- *Australia*: Australian Government, National Health and Medical Research Centre [NHMRC] Policy on the Care and Use of Non-Human Primates for Scientific Purposes 2003. Animal Research Review Panel [ARRP] Guidelines for the Housing Guinea Pigs in Scientific Institutions: Guideline 21; 2006. Victorian Codes of Practice: The Prevention of Cruelty to Animals Act (1986) (The Act) and Regulations (1997): Code of Practice for the Housing and Care of Mice, Rats, Guinea pigs and Rabbits, Appendix I.
- *United Kingdom*: the National Centre for the Replacement, Refinement and Reduction of Animals in Research [NC3Rs] Guidelines: Primate Accommodation, Care and Use (2006).

Furthermore, additional suggestions and ways of how to improve the non-human primate well-being in a captive environment will be discussed due to their high level of sentiency and the complexity of housing them (Tardif et al. 2013).

2. Micro-environment

2.1 Housing

Providing a suitable and appropriate animal enclosure is necessary in order to contribute to the well-being of the animal, the quality of animal research, production, teaching or testing programs involving animals and the health and safety of the personal. A proper housing environment has to be designed and adjusted to the species or strain chosen where the physiological, physical and behavioral needs of the animal are accounted for (Victorian Codes of Practice 2004; NRC 2011). Allowing the animal to grow, mature and reproduce normally is an important factor in designing and providing the proper housing and environment (NRC 2011).

2.2 Cage and space

Materials of the cage should not be harmful to the health and welfare of the animals. No injury should be caused by the design and construction of the enclosure (Council of Europe 2006). The enclosure should be designed in such a way that each animal has sufficient space to express a wide behavioral repertoire (Victorian Codes of Practice 2004; European Directive 2010/63/EU 2010), not providing sufficient space and high stocking densities can possibly lead to endocrine stress reaction and high frequencies of aggression depending on the species (Kaiser et al. 2010). The floor space required for water/food bowls, shelter or nest boxes and enrichment devices should not be considered as available floor space per animal. The range of minimum space requirements for mice, rats, guinea pigs and NHPs are listed in the tables and chapters below. The design and choice of material can affect a number of factors such as social contact (i.e. degree of transparency, smell and noise), heat and noise conduction. Therefore, when choosing certain materials (e.g. wire mesh or solid flooring) other issues such as which type of ventilation or room temperature have to be carefully reconsidered (Victorian Codes of Practice 2004). These same factors are affected when choosing the design of the cage, for example an enclosed 'shoebox-style' cage will have different effects on temperature, ventilation and humidity than an open type design (e.g. pens) (Victorian Codes of Practice 2004). The enclosures should be able to withstand thorough cleaning and decontamination. Regular and efficient cleaning schedules for both the rooms and enclosures are necessary. The enclosure floors should be appropriate to the age and species of the animal and should enable caretakers to remove feces (European Directive 2010/63/EU).

2.3 Bedding and nesting material

Depending on the species, appropriate bedding and nesting material and/or sleeping structures should be provided. In addition, a solid clean and comfortable resting area should always be present for the animals in the enclosure. In case of breeding animals, nesting material or structures should be provided (Victorian Codes of Practice 1997; European Directive 2010/63/EU). Providing bedding material will absorb and contain urine and feces (Blom et al 1996). It also provides a comfortable resting surface and enables several behaviors of rodents such as digging, chewing, nesting and hiding (Boyd 1988). Note that the bedding material influences the micro-environment. Differences in ammonia levels with regard to different types of bedding in mice have been observed (Smith et al. 2004). On the other hand, next to the amount and type of bedding material, other factors such as ventilation, relative humidity and temperature also affect the ammonia levels (Gonder and Laber 2007; NRC 2011).

2.4 Enrichment

The use of environmental enrichment to improve the well-being of animals has been used and incorporated world widely by for example; the European, American, and Australian legislation (Victorian Codes of Practice 2004; European Directive 2010/63/EU; NRC 2011). Environmental enrichments are aimed to provide a more complex environment which enhances species-specific behavior, promoting physical health and decreases abnormal behavior as much as possible (Newberry 1995; Baumans 2005; Baumans et al. 2010b; 2013). Environments that are not up to standards and fail to meet the animals' needs may result in the development of abnormal behavior, disorders, physiologic dysfunction and abnormal brain development (Van Praag et al 2000; Würbel 2001; Garner 2005; NRC 2011). More importantly, animals showing these types of changes will disrupt the validity, reliability and reproducibility of the research (Garner et al. 2005). For that reason it is important to enrich the primary animal enclosure to ensure proper scientific research standards and the welfare of the animal. Barren environments should be avoided at all times except when experimental set-ups require it differently and compelling scientific evidence supports this. Furthermore, enrichment is especially important for single housed animals or aggressive males (Victorian Codes of Practice 2004).

2.5 Social housing

Gregarious species should be socially housed and living in groups or pairs as long as the groups or pairs are stable, harmonious and in concordance with the experimental set-up. If individual housing has to take place because of veterinary or welfare grounds than additional resources have to be added to the care and welfare of these animals (Council of Europe 2006). Furthermore, single housed animals will spend more time on other non-social behaviors (e.g. self-directed behavior, food intake) due to the prevention of social contact which can lead to weight gain (Morgan and Einan 1976) or reduced social tolerance (Brain et al. 1980; Hurst et al. 1997). Creating stable social groups can be difficult and complex and severe forms of aggression can occur (i.e. males from certain mice strains or non-human primates). Only for that reason should animals be housed individually (i.e. next to other experimental reasons such as infectious diseases or metabolism research (Wolfensohn and Honess 2005b) as this is considered very stressful and impairs the well-being of the animal and prevents thermoregulation (Council of Europe 2006; NRC 2011; Baumans et al. 2013; Victorian Codes of Practice). Furthermore, when individual housing has to take place due to experimental or health reasons, the duration should be minimized and visual, olfactory, auditory and possibly tactile contact shall be maintained (European Directive 2010/63/EU). In addition, animals which are individually housed should have enrichments present within their enclosure (Victorian Codes of Practice 2004). Establishing harmonious and compatible groups is influenced by a number of factors such as age, sex and hierarchical rank which can occasionally be managed by good husbandry practices (Baumans et al. 2013). With group or pair housing, enough space and complexity (i.e. shelters/refuges) should be provided as animals should be able to escape aggression which can occur (Victorian Codes of Practice 2004; NRC 2011). It is recommended to keep the group composition stable once natural hierarchies and harmonious groups have been established (Victorian Codes of Practice 2004; Council of Europe 2006). Also, (re-)introduction of animals into established stable groups should be carefully monitored by trained staff (Council of Europe 2006). Besides the improved welfare, group housing utilizes the given space more efficiently where group animals share space and each animal will require less floor space with increasing group size. Thus larger groups may be housed at slightly higher stocking densities than smaller groups or individual animals (NRC 2011).

2.6 Cleaning

Adequate routines for the cleaning, washing, decontamination and, when necessary, sterilization of the animal enclosures and accessories (e.g. bottles) are necessary in order to maintain a high standard of cleanliness and proper hygiene. The cleaning and disinfection of the enclosures should not harm the health or welfare of the animal. When changing the bedding material of the enclosure clear operating routines should be in place (European Directive 2010/63/EU; NRC 2011). Furthermore, cleaning and renewal of the material which covers the floor (e.g. bedding and nesting material) of the enclosure should be carried out on a regular basis in order to maintain high cleaning standards and to avoid the bedding material to become a source of infection and parasite infestation (Council of Europe 2006; NRC 2011). Soiled bedding should be replaced with fresh material as often as necessary to keep the animals clean and dry. Furthermore, an increase in ammonia levels can be caused by not replacing soiled bedding which in turn can irritate the mucous membranes of animals (NRC 2011). Cleaning routines must be carefully adjusted to the species and their behavioral needs as cleaning will always cause some type of social disruptions (e.g. odor-marking in some species will be disturbed by cleaning). Furthermore, creating the cleaning schedule/routine has to be discussed by the investigator and the animal care personnel. There is not a set number of times in which the enclosure has to be cleaned. However, it typically ranges from daily to weekly cleanings. The type of animal enclosure, the type of animal, the stocking density, relative humidity, temperatures and the ability of the ventilation system to maintain suitable air quality should be taken into account when cleaning schedules are formed (European Directive 2010/63/EU; NRC 2011).

3. Mice

3.1 Cage and space requirements

The minimum space requirements regarding mice are listed in Table 1. These values are ranges of the minimum space requirements based on the documents listed in the table description. The enclosure height is the vertical distance between the floor and top of the enclosure. Mice must be able to stand upright on hind legs and if possible be able to look outside of the enclosure, especially when they are single housed (Victorian Codes of Practice 2004). The space requirements should be maintained at all times, exceptions can be made for short-term housing (i.e. no more than a day). Long-term procedures or experiments should take potential growth of the animals into consideration regarding proper space requirements (European Directive 2010/63/EU; NRC 2011). Under the circumstances that the enclosures are larger (i.e. 950 to 1500 cm²) and adequate enrichment is provided, post-weaned mice can be kept under higher densities with smaller floor area per animal but only for a limited period of time (Council of Europe 2006). Also, the welfare of the animals cannot be compromised and aggressive behavior, abnormal behavior, health and physiological or behavioral stress responses should be monitored (Council of Europe 2006; NRC 2011).

Table 1. Range of minimum enclosure dimensions and space requirements of mice. Requirements were based on the European Directive Appendix III (2006); The National Research Council (2011); Victorian Code of Practice (2004) and the Canadian Council on Animal Care (2003).

Single or group housing or breeding animals	Body weight (g)	Floor area per animal (cm²)	Enclosure height (cm)	Minimum cage size (cm²)
Single	any	200	12-15	
Group	<10 10 – 20 20 – 30 30 – 40 >40	38.7 – 65 60 – 77.4 60 – 100 70 – 100 96.7 – 100	12-15	330
Breeding	Pair Extra females + litter	300 – 330 For each additional female + litter 150 – 180 shall be added.	12-15	330
Post-weaned stock at breeders (950).	<20	40	12	950
Post-weaned stock at breeders (1500)	<20	30	12	1500

3.2 Floors

Solid floors or perforated floors, preferably not wire mesh or grid floors, should be used when housing mice. If wire mesh or grid floors are used than a solid (e.g. solid mat) or bedded area for the animals to rest should be provided unless the experimental specifications require it differently. Furthermore, these types of flooring can lead to serious injuries (Fullerton and Gilliat 1967; NRC 2011). Therefore, if wire mesh or grid floors are chosen, thorough and regular inspections of the floor and animals are necessary.

3.3 Bedding and nesting material

In order to provide a living environment where mice can express certain natural behaviors such as huddling, hiding, resting and breeding, bedding and nesting material should be provided to the animals regardless whether they are in stock, breeding or under procedures (Latham and Mason 2004). In addition, cleaning and sanitation are facilitated by the absorption of feces and urine. To date wood shavings or wood chips, paper or other materials have been used as bedding materials for laboratory mice. The characteristics of good bedding material will enable the mice to easily hide, build nests and should be soft (Ago et al. 2002). Large paper fibers fulfill these requirements and were preferred by mice out of four types of bedding (Ago et al. 2002). Cloth bedding (Agrebe™) was proposed by Kawakami et al. (2007) as the alternative for paper and wood chip bedding as concerns arise about protecting the environment and natural resources and cloth bedding is reusable. Furthermore, the cloth bedding was preferred by the mice over paper and wood chip bedding.

3.4 Enrichment

Next to bedding material, providing nesting material is an important enrichment to ensure the mice well-being. In a preference test it was shown that given the choice, mice preferred tissue nesting material on grid floor (which has been avoided before) over an enclosure which contained saw dust bedding material with a metal nest box which displays the importance of nesting material with regards to the animal's welfare and comfort (Van de Weerd et al. 1998). The profound effect of not providing nesting material was shown in a study where mice (from the age of 3 weeks) were brought up without nesting material. These mice showed a significant increase in anxiety-like behavior and learning and memory were negatively affected (Kulesskaya et al. 2011). In addition Van Loo et al. (2001) found a decrease in inter-male aggression when nesting material was provided.
Regarding the type of nesting material, paper derived materials such as tissues, towels and paper stripes were preferred over wood-derived material. However, the structure (i.e. ability to build nests) of the material was more important than the exact type (Van de Weerd et al. 1998). Paper toweling was eagerly used by non-breeding female mice as nesting material which can be an inexpensive way to provide nesting material and improve the well-being of mice (Sherwin 1997).

Besides nesting material, mice also benefit from enrichment which enable foraging, exploring and social behaviors. Therefore, providing wood sticks, cardboard, plastic tubes and opportunities to find food (e.g. hard shelled nuts, sunflower and/or sesame seeds) and social interaction will improve the welfare of the animal (Victorian Codes of Practice 2004; Hutchinson et al. 2005; Council of Europe 2006; Baumans et al. 2013). When dealing with individually housed or aggressive mice, providing an exercise-wheel should be considered (Victorian Codes of Practice 2004). Animals are motivated to perform wheel running behavior and Shermin (1998) suggests this is perceived by animals as a highly important and self-reinforcing activity (Collier et al. 1990). However, the use of the exercise-wheel has to be carefully monitored and perhaps provided only a limited amount of time each day as running in the wheel can sometimes develop into maladaptive behavior or stereotypic activity (Sherwin 1998). It has been shown with rats that unlimited access to a running-wheel can lead to such an intense use that substantial decrease in body weight occurs (Routtenberg and Kuznesof 1967). Not only can this interfere with the experimental set-up but it can also be dangerous for the animals.

3.5 Social housing

Mice are gregarious species and should be socially housed in groups or pairs instead of individual housing. Individual housing is considered stressful for mice, however, certain strains (e.g. BALB/c or FVB) are known to be aggressive and problematic to socially house, especially the (reproductive) males (Laboratory Animal Science Association 1998). Next to the males, aggressive females (i.e. nest defense) have been reported when pregnant and lactating (Council of Europe 2006). In mildly aggressive strains or when the mice are young, socially stable groups can be formed (Bisazza 1981; Van Loo et al. 2004). It is advised to wean animals into social groups in order to prevent fighting and increase the chance of creating stable, harmonious and manageable hierarchies (Victorian Codes of Practice 2004). The importance of social contact was shown by giving mice a choice between a cage containing tissue nesting material or a cage which was near a familiar cage mate. They preferred spending their time and sleeping in close proximity of a familiar cage mate instead of using nesting material. Furthermore the need of social contact increased with age. It was suggested that in case of individual housing, provision of nesting material could partly compensate for the deprivation of social contact (Van Loo et al. 2004a). At times of surgery procedures, mice are usually housed individually when they are coming out of surgery. However, social housing after surgery is more beneficial; socially housed mice had overall lower heart rates, ate less food and differed less in behavior pre- and post-surgery (Van Loo et al. 2007). Still if social housing after surgery procedures is not possible than individual housing is advised instead of housing near a cage mate and having visual, olfactory and/or auditory contact (i.e. separation by a grid or mesh wall) since these mice had the highest overall heart rate which is considered stressful (Van Loo et al. 2007).

4. Rats

4.1 Cage and space requirements

The minimum space requirements for rats are listed in Table 2. These values are ranges of the minimum space requirements based on the documents listed in the table description. The enclosure height is the vertical distance between the floor and top of the enclosure. Rats must be able to stand upright on hind legs and if possible be able to look outside of the enclosure, especially when single housed (Victorian Codes of Practice 2004). The space requirements should be maintained at all times, exceptions can be made for short-term housing (i.e. no more than a day). Long-term procedures or experiments should take potential growth of the animals into consideration regarding proper space requirements (European Directive 2010/63/EU; NRC 2011). Under the circumstances that the enclosures are larger (i.e. 1500 to 2500 cm²) and adequate enrichment is provided, post-weaned rats can be kept under higher densities with smaller floor area per animal but only for a limited period of time (Council of Europe 2006). Also, the welfare of the animals cannot be compromised and aggressive behavior, abnormal behavior, health and physiological or behavioral stress responses should be monitored (Council of Europe 2006; NRC 2011).

Table 2. Range of minimum enclosure dimensions and space requirements for rats. Requirements were based on the European Directive Appendix III (2006); National Research Council (2011); Victorian Code of Practice (2004) and the Canadian Council on Animal Care (2003).

Single, group housing or breeding animals	Body weight (g)	Floor area per animal (cm²)	Enclosure height (cm)	Minimum cage size (cm²)
Single	<250 250 – 550 >550	500 700 800	17.8 – 18	
Group	<100 100 – 200 200 – 300 300 – 400 400 – 500 >500	109.6 – 200 148.4 – 225 187.1 – 250 250 – 350 250 – 450 250 – 600	17.8 – 18	800 800 800 800 800 1500
Breeding	Female + litter Extra adult	800 For each additional adult 400 shall be added.	17.8 – 18	800
Post-weaned stock at breeders (1500).	<50 50 – 100 100 – 150	100 125 150	17.8 – 18	1500 1500 1500

	150 – 200	175		1500
Post-weaned stock at breeders (2500)	<100 100 – 150 150 – 200	100 125 150	17.8 – 18	2500 2500 2500

4.2 Floors

Similar requirements with regards to the flooring for mice apply to the flooring for rat enclosures. Solid floors or perforated floors, preferably not wire mesh or grid floors, should be used when housing rats. Particularly during rest, rats prefer solid floors over wired ones (Manser et al. 1995). If wire mesh or grid floors are used than a solid (e.g. solid mat) or bedded area for the animals to rest should be provided unless the experimental specifications require it differently. Furthermore, these types of flooring can lead to serious injuries (Fullerton and Gilliat 1967; Raynor et al. 1983; NRC 2011). Therefore, if wire mesh or grid floors are chosen, thorough and regular inspection of the floor and animals are necessary.

4.3 Bedding and nesting material

As mentioned before in the 'bedding and nesting material' section on mice, providing proper bedding and nesting material is a crucial aspect to the welfare of the animal. Not only does it contribute to the expression of several natural behaviors of the rat, it also facilitates sanitation and cleaning of the enclosure (NRC 2011) and influences the ammonia levels (Smith et al. 2004; Gonder and Laber 2007). Like mice, nest building is common for rats and observed in wild and pet rats when nesting material is provided (Van Loo and Baumans 2004). However, there is a difference between mice and rats regarding the use of nesting material. When nesting material is provided to rats for the first time in their adult life, their initial response is to chew and eat it. Rats need to learn how to build nests from their mother and when they do, providing nesting material is a suitable type of environmental enrichment. Furthermore, the type of nesting material can help in avoiding chewing and eating of it, for example Enviro-dri (i.e. paper-fibers) was eaten less often than Kleenex tissues (Van Loo and Baumans 2004). Long paper-fibers as nesting material were also preferred in a study by Manser et al. (1998) where six different choices were given.

Next to nesting material, providing a nesting box should be considered a suitable and important enrichment for rats. When given the choice between shredded paper (nesting material) and a tin nesting box, rats showed a preference towards the nesting box (Van Loo and Baumans 2004). In addition, a strong preference was found for nesting boxes that limit incoming light (Manser et al. 1998). With regards to bedding material, rats prefer the type of bedding on which they were raised except for corn-cob bedding (Ras et al. 2002). Studies have shown a preference for wood shavings (i.e. aspen chips) over corn-cob bedding for rats (Blom et al. 1996; Ras et al. 2002; Krohn and Hansen 2008). Only when air quality is a significant problem, then corn-cob bedding can be a valuable option as bedding material (Ras et al. 2002).

4.4 Enrichment

Providing enrichment for rats can have beneficial effects on aggression in a group. Group housed rats in the enriched condition (i.e. rope, ladder, crawl ball, shelter etc.) showed lower levels of agonistic behavior compared to rats living in non-enriched cages (Abu-Ismail et al. 2010). Tubes, boxes and/or pipes will make the environment more complex and enable foraging and hiding opportunities. In addition, PVC pipes should be given to individually housed or aggressive rats (Victorian Codes of Practice 2004). As mentioned before, wood sticks can be used as enrichment for chewing and gnawing (Council of Europe 2006; Kaiser et al. 2010). Providing pumpkin seeds can function as foraging enrichment as well (Victorian Codes of Practice 2004).

4.5 Social housing

Rats are social animals and their social needs are comparable with mice. It is recommended they are group or pair housed instead of individually housed. Rats are typically nonaggressive and are therefore more easily socially housed (Hutchinson et al. 2005). Single housed rats show more self-directed behavior, reduced activity and behaviors linked to escape and search of social information than group housed rats (Hurst et al. 1997). Furthermore, rats who have been isolated and then re-grouped show more play and social behaviors (Varlinskaya et al. 1999). Rats should be weaned into groups in order to prevent fighting and create stable manageable hierarchies (Victorian Codes of Practice 2004).

5. Guinea pigs

5.1 Cage and space requirements

The minimum space requirements are listed in Table 3. These values are ranges of the minimum space requirements based on the documents listed in the table description. The enclosure height is the vertical distance between the floor and top of the enclosure. The guinea pig should be able to stand upright on hind legs (Kaiser et al. 2010). Preferably, the minimum height of 23 cm should be used as it enables juvenile guinea pigs in expressing playful behavior (e.g. frisky hops) and adult guinea pigs can fully stand on hind legs at this height (Kaiser et al. 2010). In order for the animals to optimize the floor space provided, appropriate shelters should be placed within the enclosure (Reinhardt 2002). The space requirements should be maintained at all times, exceptions can be made for short-term housing.

When housing guinea pigs in large pens it is important to provide open sides or wire mesh sides, next to closed sides (e.g. opaque materials or metal), to enable the guinea pigs to see the people approaching but also provide hiding opportunities (ARRP 2006; Kaiser et al. 2010). Sudden noises or unexpected movements could cause these animals to panic and stampede and possible injuries could arise (CCAC 1984; ARRP 2006). Even though it is thought that the use of floor pens are taking up to much space and are difficult to thoroughly clean, they are inexpensive, easy to construct and flexible (CCAC 1984). If cages are preferred than it is not recommended to use only transparent or translucent materials or wire mesh cages. Partial transparent or wire mesh sides of a cage can be used as this will allow the animals to see people approaching (Scharmann 1991; ARRP 2006).

Table 3. Range of minimum enclosure dimensions and space requirements of guinea pigs. Requirements were based on the European Directive Appendix III (2006); National Research Council (2011); Australian Animal Research Review Panel (2006) and the Canadian Council on Animal Care (2003).

Single, group housing or breeding animals	Body weight (g)	Floor area per animal (cm^2)	Enclosure height (cm)	Minimum cage size (cm^2)
Single	<250 250 – 550 >550	300 – 700 650 – 900 650 – 1000	18 – 20 22 – 23 22 – 23	
Group	<200 200 – 350 350 – 550 550 – 700 >700	200 – 500 387 – 500 450 – 800 600 – 800 600 – 900	17.8 – 23	1800 1800 1800 2500 2500
Breeding	Female + litter	1200	23	

	Breeding pair + litter	2500	23	2500
	Extra female	For each additional female in harem add 1000	23	

5.2 Floors

Similar requirements with regards to flooring for mice and rats apply to the flooring for guinea pig enclosures. Solid floors or perforated floors, preferably not wire mesh or grid floors should be used when housing guinea pigs. Especially when housing breeding animals or for long-term purposes, wire floors are not suitable and solid floors are necessary (Ediger 1976; Kaiser et al. 2010). If wire mesh or grid floors are used then at least a solid (e.g. solid mat) or bedded area for the animals to rest should be provided unless the experimental set-up require it differently (Kaiser et al. 2010). Furthermore, these types of flooring (i.e. grid and wire floors) can lead to serious injuries (Fullerton and Gilliat 1967; Raynor et al. 1983; NRC 2011). Therefore, if wire mesh or grid floors are chosen, thorough and regular inspection of the floor and animals are necessary. Also, advise on requirements of using wire mesh floors exist and are based on the weight of the animal. Guinea pigs under 350 g require a mesh of approximately 10 mm (3/8 in) made of 10-12 gauge wire; larger animals need a 16 mm (5/8 in) mesh of 9-10 gauge (CCAC 1984).

5.3 Bedding and nesting material

When housing guinea pigs, wood shavings or –chips are frequently used and satisfactory for bedding (CCAC 1984; Kaiser et al. 2010). It is not recommended to use sawdust alone or any other small particle type of bedding due to the possibility of it adhering to the vulva, scrotum and prepuce which can cause irritation and obstruction resulting in reduction of fertility (CCAC 1984; Anderson 1987). A nest box or any other type of shelter or refuge is necessary to house guinea pigs, especially when females have to give birth (ARRP 2006). Unlike rats and mice, guinea pigs do not burrow but may use burrows from other animals in the wild (Kaiser et al. 2010; Baumans et al. 2013). Tubes, pipes (e.g. PVC) or shelters have to be present in the cage or pen to allow the animals to climb onto or hide under them as they are easily frightened (ARRP 2006; Kaiser et al. 2010; Baumans et al. 2013). When using piping as refuge, these must be short enough to prevent more than two guinea pigs inside the piping. Allowing more than two animals inside the pipe could cause the animals in the middle to be smothered and suffocated (Kaiser et al. 2010).

5.4 Enrichment

The most important enrichment for the guinea pig is the social group and social interaction, see ‘social housing’. In addition to the provision of shelters/refuges. It is recommended to provide hay to satisfy the need for roughage and concealment (Council of Europe 2006; Baumans et al. 2013). Providing enough hay or placing hay in hay racks positioned just above the floor, or giving Lucerne hay biscuits enables tunneling and could function as temporary shelter or nest box if needed (ARRP 2006). Furthermore, the presence of hay may help in preventing behavior problems such as hair damage caused by barbering and will assist in maintaining tooth care (Anderson 1987; ARRP 2006). Hay must be renewed frequently though, dirty or mouldy hay is not beneficial for the welfare if the animals. With regards to chewing and gnawing, wood sticks, small softwood blocks and/or pop sticks can be used as enrichment (Scharmann 1991; Council of Europe 2006; Kaiser et al. 2010).

5.5 Social housing

Guinea pigs are very social and use a relatively complex variety of vocalizations along with scent marking for communication (Hutchinson et al.2005). They should be housed with social partners either in pairs (i.e. male-male or female-male), in harems or in female groups (Kaiser et al. 2010). Group size should be around 5-10 animals (ARRP 2006). Female groups can be of unlimited size depending on the enclosure size (Kaiser et al 2010). Group housing is more beneficial than single housing as male guinea pigs housed in groups can cope more effectively with stress situations than single housed males (Sachser et al 1994). By allowing a social group to grow and evolve naturally a social hierarchy will establish and breeding group size can be larger (e.g. 3-10 males housed together with 15-30 females) (ARRP 2006). Males can be kept into groups up to 4 months of age and after that should be housed in pairs (ARRP 2006; Kaiser et al. 2010; Baumans et al. 2013). No more than two males should be housed together (Kaiser et al. 2010). Aggression can occur among males, especially in the presence of an oestrus female or if more than two males are housed together (ARRP 2006; Kaiser et al. 2010). Aggression can also occur among females when living in harems. However, this is usually of low intensity and rare (Kaiser et al. 2010). Early social stress (i.e. mothers experiencing social stress) should be avoided at all times since this can induce changes in the endocrine, autonomic and limbic brain function and be expressed in male and female social behavior (i.e. the offspring) (Kaiser et al. 2003; 2010).

Table 4. Overview and summary of rodent enrichments.

Species	**Enrichment**
Mice	Nesting material, shelters/refuges, running wheel, cardboard, (PVC) pipes, wood sticks, hard shelled nuts, sunflower seeds, sesame seeds
Rats	Nesting material, tubes, boxes, (PVC) pipes, crawl balls, rope ladders, shelters/refuges, pumpkin seeds, wood sticks
Guinea pigs	Shelters/refuges, (PVC) pipes, hay, wood sticks, softwood blocks, pop sticks

6. Non-human primates

6.1 Cage and space requirements

Providing sufficient space is very important for non-human primates. Not only does it influence behavior, it also offers opportunities for providing enrichment and housing animals in social harmonious groups (Prescott and Buchanan-Smith 2004). The minimum space requirements for several non-human primate species are divided into single and group/pair housing guidelines and listed in Tables 4, 5 and 6. Guidelines from several countries differ greatly on space requirements and choice of calculation. For example the European Directive (2010/63/EU) based their space requirements on the age of the animals rather than their weight like the Canadian Council on Animal Care (2003) and the National Research Centre (2011) did. This makes it difficult to summarize all the different guidelines into one universal guideline. It has been brought up that not a single factor such as weight or age can be satisfactory in order to calculate suitable cage dimensions. Morphometric, ecological, social and behavioral characteristics should be used instead (Prescott and Buchanan-Smith 2004).

With regards to baboon and macaque species, both single and pair/group housing requirements are given. The amount of space should enable the animal to carry out their normal locomotor activity and behavioral repertoire (e.g. climbing, running, resting, leaping, foraging, etc.) (NC3Rs 2006) and discourage abnormal behavior (Council of Europe 2006). Regardless of the type of housing, the minimum cage height (i.e. from cage floor to cage top) of the enclosure should enable the animal to stand erect with their feet on the floor (NRC 2011). However, horizontal and vertical space requirements will differ in importance regarding different non-human primates. Vertical space is more important for arboreal and neo-tropical species such as marmosets and macaques than baboons where horizontal space is more useful and important. Their flight reaction will be vertical, derived from the wild where fleeing or taking refuge from predators is sought in trees. Therefore, the enclosure height should be suitable to allow the animal to flee and perch at a sufficient height for it to feel secure and its tail not touching the floor which will improve their well-being (Clarence et al. 2006; Council of Europe 2006). To enable full utilization of the vertical space, climbing frames/structures, perches and/or nesting boxes should be provided within the enclosure (CCAC 1984; NHMRC 2003). The use of double-tiered cage should be avoided since they restrict the amount of vertical space available to the animal (NC3Rs 2006; Council of Europe 2006). In addition, it has been shown with rhesus and long-tailed macaques that a strong preference for elevated areas exist (i.e. the upper cage versus the bottom cage) regardless of lighting differences (MacLean et al. 2009).

Metal is most commonly used as the cage material. However, other materials such as glass, wood or laminates provide satisfactory as well and provide a quieter environment (Council of Europe 2006). If the walls include wired mesh then, even though this may provide climbing opportunities, sufficient diagonal branches or perches should be provided (Council of Europe2006). Furthermore, when wired mesh is used, the size of the mesh openings should not allow limbs to get trapped and careful choice of the type of mesh should be taken (CCAC 1984; Council of Europe 2006).

6.1.1 Single housing requirements

The minimum space requirements for housing baboons and macaques individually are listed in Table 5. These values are ranges of the minimum space requirements based on the documents listed in the table description. Preferences should be made for social housing and enclosure dimensions of Table 7.

Table 5. Range of minimum space requirements for single housing regarding baboon and macaque species. Minimum values are based on the Canadian Council on Animal Care (2003).

Species	Weight (kg)	Floor area/animal (m^2)	Height (m)
Baboons	5-12 >12	0,74 1,39	0,91 1,22
Macaques	< 7 7-15 >15	0,4 0,6 0,75	0,81 0,91 1,20

The minimum space requirements for marmosets, tamarins and squirrel monkeys when housed individually or in groups or pairs are listed in Table 6. The top of the enclosure should be at least 1,8 meters above the floor (European Directive 2010/63/EU).

Table 6. Range of minimum space requirements for group or pair housing regarding marmosets, tamarins and squirrel monkeys. Minimum values are based on the European Directive (2010/63/EU).

Species	Minimum floor area of enclosures for 1 or 2 animals plus offspring up to 5 months old (m^2)	Minimum volume per additional animal over 5 months (m^3)	Minimum enclosure height (m)
Marmosets	0,5	0,2	1,5
Tamarins	1,5	0,2	1,5
	Minimum floor area for 1 or 2 animals (m^2)	Minimum volume per additional animal over 6 months of age (m^3)	Minimum enclosure height (m)
Squirrel monkeys	2,0	0,5	1,8

6.1.2 Group or pair housing requirements

Below are the minimum space requirements for macaques, vervets, baboons and chimpanzees when housed in groups or pairs (Table 7). With regards to macaques and vervets, the space requirements may hold up to three animals if they are less than three years of age. The enclosure dimensions may hold up to two animals if they are older than three years. Furthermore, no additional space/volume is required when macaques or vervets younger than two years of age are housed together with their mother in breeding colonies. Concerning baboons, these space requirements may hold up to two animals within the enclosure. Also, no additional space/volume is required when baboons younger than two years of age are housed together with their mother in breeding colonies.

Table 7. Range of minimum space requirements for group or pair housing regarding macaques, vervets, baboons and chimpanzees. Minimum range values are based on the Canadian Council on Animal Care (1984) and European Directive (2010/63/EU).

Species	Minimum enclosure size (m^2)	Minimum enclosure volume (m^3)	Minimum volume per animal (m^3)	Minimum enclosure height (m)
Macaques and vervets				
Animals less than 3 yrs of age	2,0 – 3,0	3,6	1,0	1,8
Animals from 3 yrs of age	2,0 – 3,0	3,6	1,8	1,8
Animals held for breeding purposes			3,5	2,0
Baboons				
Animals less than 4 yrs of age	2,8 - 4,0	7,2	3,0	1,52 – 1,8
Animals from 4 yrs of age	2,8 - 7,0	12,6	6,0	1,52 – 1,8
Animals held for breeding purposes			12,0	2,0
Chimpanzees				
Juveniles (<10kg) Adults (>10kg)	1.4 ≥2,32			1,52 2,13

6.2 Outdoor enclosures

Non-human primates should have access to an outdoor enclosure where possible (NHMRC 2006; Council of Europe 2006). No space requirements or dimensions are needed as this is simply extra space available to the animals. The outdoor enclosure should have a rooftop to protect the animals from the sun or rain and allow them to go outside regardless of the weather circumstances. If there is no rooftop present then adequate opportunities for sheltering should be available (Council of Europe 2006). Outdoor enclosures are usually made out of metal but other materials such as wood can also be used if it is weather-proofed. Also, if wood is chosen, which is advised as it is a more natural and more silent material than metal, the animals must not be able to chew it or it must be protected with wired mesh and a non-toxic treatment. The floor of the outdoor enclosure can be either concrete or natural vegetation. A concrete base should be covered with a non-toxic substrate. Indoor heating should be present since animals will utilize the outdoor enclosures in wintertime. At least two corridors/doors should be made available as a connection between the in-and outdoor facility due to problems which can occur between dominant and subordinate animals when one corridor/door is present (Council of Europe 2006).

6.3 Floors

The selection of good flooring can encourage arboreal species to make more use of the floor space provided. Solid flooring is mostly recommended when housing non-human primates (Rennie and Buchanan-Smith 2006). However, this type of flooring is most important for terrestrial Old World primates such as baboons, macaques and chimpanzees who spend most of their time on the ground (Hardy et al. 2004). Grid or wire mesh floors should only be used if there is any justification for it such as compelling scientific reasons or veterinary motivation. The reason for this is that no substrate can be placed on grid floors which diminishes comfort for the animals as well as eradicating the opportunity for a simple foraging enrichments (i.e. scattering food between the substrate on the floor) (Council of Europe 2006; NC3Rs 2006). If wire mesh or grid floors are used then the size of the wire mesh openings should be large enough for feces to pass through yet small enough to prevent limbs getting caught (CCAC 1984). When housing arboreal New World primates whose hand and feet are adapted for grasping branches and twigs, wire mesh or grid floors can be used. Captive bred marmosets preferred wired mesh floor over a solid floor covered with sawdust by spending more time on the wired mesh floor and making more visits than the solid floor (Hardy et al. 2004). However, these marmosets were raised on wire mesh floors which could have influenced their preference. Therefore, the type of bedding on which they were raised on have to be taken into account when choosing the type of flooring.

6.4 Substrate

Substrate can and should be provided when the enclosures have solid floors as it not only provides a level of comfort as well as providing foraging opportunities (Council of Europe 2006; Rennie and Buchanan-Smith 2006). Many substrates are available and satisfactory as long as they are non-toxic (Council of Europe 2006). Wood -shavings or -chips, wood granulate with a low dust level, straw or shredded paper are all satisfactory and will promote foraging (Council of Europe 2006; Rennie and Buchanan-Smith 2006). As for the outdoor enclosures, grass, herbage wood chip or bark chip are suitable substrates. It has been shown with rhesus macaques that the type of outdoor substrate can change the activity budget and hair loss. Provided with grass, rhesus macaques had less hair loss due to less time grooming and spent more time foraging compared to gravel substrate (Beisner and Isbell 2008). Nest material such as wood wool, dry leaves or straw should be provided to some non-human primates which require this (e.g. prosimians) (Council of Europe 2006).

6.5 Enrichment

Non-human primates kept in a barren environment, that is without stimulation, continually changing and manipulatable objects will rapidly come to suffer from intellectual, sensory and motory deprivation (CCAC 1984). They are, like humans, capable of suffering from pathogenic boredom and may express this in a variety of abnormal behaviors such as coprophagy, self-mutilation or hair-plucking (McGrew 1980; CCAC 1984; Wolfensohn and Honess 2005b). Therefore, some novelty should be introduced at intervals to prevent boredom. Re-arrangement of enclosure furniture (e.g. ladders, ropes, etc.), feeding practices, varied diet or introducing new enrichments will aid in this (CCAC 1984; Council of Europe 2006; NC3Rs 2006). Another important aspect of primate enrichment is the level of predictability and control it has over its environment (Council of Europe 2006). It has been suggested that a balance between total control and predictability and lack of control and predictability is optimal (Sambrook and Buchanan-Smith 1997). Thus objects that are manipulatable and at times unpredictable are favored as enrichment for example, a simple swing or rope ladder will have a certain level of unpredictability and controllability (Markowitz and Line 1989; Buchanan-Smith 1997; Rennie and Buchanan-Smith 2006). Non-human primate enclosures should be furnished in such a way which allow the primate to express their full range of species-typical behaviors. This includes, running, resting, climbing, leaping, social interaction and foraging, depending on the species. As mentioned before, enrichments such as perches, shelves, ladders, ropes, platforms, hammocks, branches and swings will enable primates to make more use of the space provided and make it more complex and challenging (NC3Rs 2006; Council of Europe 2006; Rennie and Buchanan-Smith 2006). Regarding macaques, shelves and perches should not be placed above one another. A space between the shelve and the enclosure wall should be present as the tail of the macaque has to be able to move freely (Council of Europe 2006). Wooden perches should be used when housing marmosets, tamarins or macaques as these will enable natural behaviors such as gnawing (i.e. wooden blocks could also be used), space to groom and scent-marking (Buchanan-Smith 1997; Council of Europe 2006). In addition, nest boxes will provide hiding opportunities, give them a sense of security and retreating opportunity for the night (Poole et al. 1999) which also applies for other species such as squirrel monkeys. Even though wooden furniture has to be replaced more often than furniture made of stronger material (e.g. PVC or metal), this material should be used when housing species such as the callitrichid species as they have claw-like nails and have difficulty grasping smooth metallic or plastic surfaces (Rennie and Buchanan-Smith 2006). In addition, wooden materials in general provide a higher degree of responsivity and texture as well as dampen noise (Eckert et al. 2000; Rennie and Buchanan-Smith 2006). Also, metal or plastic nest boxes collect breath condensate which causes the fur of the animals to get wet and possibly causes the hair to fall out more easily (Poole et al. 1999). A summery and overview of all enrichments is listed in Table 8. Foraging enrichment is a very important type of enrichment which aims at extending the time the primates spend feeding. As for some species foraging and feeding takes more than 50% of their time in the wild (Anderson and Chamov 1984; Baskerville 1999). As mentioned before in the substrate section, providing substrate on solid flooring will enable foraging opportunities and is a simply efficient and economical technique (CCAC

1984; Newberry 1995; Council of Europe 2006). Furthermore, for some species (e.g. squirrel monkeys) having a solid floor with substrate will enable play behavior as well. Other foraging enrichments include feeding puzzles, non-toxic brow foliage, freezing juice or small food items in ice cubes or simply providing nuts inside their shells or fruit that has not been peeled yet (Schapiro et al. 1996; Buchanan-Smith 1997; NHMRC 2003). As for long-tailed macaques or rhesus monkeys, a water tank containing food is of particular value as they will dive into the tank to retrieve food items (Council of Europe 2006). In addition, placing food such as fruit or vegetables on top of a wire mesh roof is also a simple but effective foraging enrichment for several species; baboons; macaques and vervets (Council of Europe 2006). Regarding marmosets, foraging devices should be present in the upper part of the enclosure as they are reluctant to spend time in the lower part. Depending on the type of flooring, wood chips on a solid flooring will encourage foraging as spilled food will fall down on the floor area. If wire mesh is chosen than additional foraging enrichment should be present. Dowels drilled with holes and filled with gum Arabic have proved to be a successful and suitable foraging enrichment for marmosets (Council of Europe 2006). Next to foraging enrichments, provision of toys, chews, tactile and destructible materials (e.g. cardboard boxes) will aid in the prevention of boredom and abnormal behaviors (Jennings and Prescott 2009). The presence of conspecifics is the most effective enrichment out of all types (Reinhardt 1990; Schapiro et al. 1996; Rennie and Buchanan-Smith 2006). Visual barriers, which allow the animals to be out of sight of one another, should be present when the animals are group housed (Council of Europe 2006). Regarding single housed primates, they should never be completely isolated from conspecifics or any form of social interaction. Visual, auditory, olfactory and possible tactile access should be maintained. Especially tactile access should be provided as primates in adjacent cages have been observed to groom each other or hold hands (Brinkman 1996; Crockett et al. 1997; Rennie and Buchanan-Smith 2006). Interaction with the human caretaker can be seen as a form of social enrichment as a strong, positive bond can develop with regular care takers and the primates (Reinhardt 1997). Positive interactions with caretakers can reduce abnormal behavior, promote species-typical behavior and improve the well-being of the animal (Reinhardt 1997; Baker 2004; Bayne 2002; Waitt et al. 2002; Coleman 2011), see also the discussion.

6.6 Social housing

Non-human primates should be socially housed by default (CCAC 1984; NHMRC 2003; Council of Europe 2006; NC3Rs 2006; NRC 2011), as social deprivation and/or individual housing are considered major stressors and have an influential factor in the development of abnormal behaviors such as hair-plucking or self-biting (Harlow and Harlow 1962; Novak and Suomi 1991; Bayne 1992; Jennings and Prescott 2009). For example, the longer macaques were kept apart from the group and singly-housed the larger the chance of developing abnormal behavior (Lutz et al. 2003). Social interaction is considered one of the most important factor influencing the well-being of non-human primates (NC3Rs 2006). However, species specific difference exist in the optimal social grouping (Novak and Suomi 1991; Rennie and Buchanan-Smith 2009). The NHPs should be housed either in compatible pairs or lager groups of compatible animals (NRC 2011). A trained person with knowledge of primate behavior (e.g. primate behavior ecologist/specialist) should be available and perhaps part of the staff in order to offer advice on the social behavior, management and enrichment strategies of primates (Council of Europe 2006; Jennings and Prescott 2009). When creating harmonious and compatible groups, the natural organization (i.e. male-female ratios, dominance hierarchies, breeding requirements social behavior patterns) of the species should be taken into account (CCAC 1984; Council of Europe 2006; NRC 2011). Housing should be designed in such a way that the impact of aggressive encounters is minimized (NHMRC 2003). Visual barriers and multiple escape routes should be present when housing NHPs in groups to enable animals to escape and/or hide from each other and to prevent aggressive escalations between dominant and subordinate animals (Council of Europe 2006; NC3Rs 2006). In addition, dominant animals should not be able to control food and water resources or to exclude subordinate animals from different areas (NC3Rs 2006). Careful monitoring of aggressive encounters should take place when creating new groups, mixing of groups or introducing a new member of the group (Council of Europe 2006; NC3Rs 2006). When NHPs have to be single housed, under close supervision, due to exceptional veterinarian reasons or scientific reasons the duration should be as short as possible. Additional resources such as environmental enrichment and extra space should be provided for single housed NHPs (NC3Rs 2006; Council of Europe 2006; NRC 2011). Furthermore, when group housing is not possible, same sex pair housing is preferred and a good alternative for single housing (Rennie and Buchanan-Smith 2006). This type of housing is preferred over single housing as it can decrease the occurrence of abnormal behavior (e.g. decline of self-biting in rhesus macaques when housed in same sex pairs (Reinhardt 1999)). The same sex pairs have to be compatible and cannot be housed too closely next to the opposite sex as this can lead to aggression in males (Council of Europe 2006).

6.7 Weaning

Natural weaning should be preferred as early weaning can cause behavioral and physiological signs of distress. For example, weight loss, depression related behavior, rise in cortisol were observed in early weaned rhesus macaques (Seay et al. 1962; Koyama and Terao 1986). Separation of the infant from a colony causes distress to both the young and mother. Therefore, it is very important to leave the young within their colony until they are independent (Council of Europe 2006). Young non-human primates should not be separated from their mother until they have reached the age of six to twelve months depending on the species. Regarding baboons, vervets, marmosets and tamarins, they should not be separated from their mother before the age of eight months (European Directive 2010/63/EU). Eight months is satisfactory for macaques as well but twelve months is preferred (Jennings and Prescott 2009). For squirrel monkeys, separation from their mother should not take place before the age of six months.

Table 8. Overview of non-human primate enrichments.

Type of enrichments	Examples of enrichment devices
Cage	Perches, shelves, ladders, ropes, platforms, hammocks, branches, swings, nest boxes, tires, barrels
Food-Foraging	Feeding puzzles, food between substrate, ice-cubes, frozen juice, water tank, dowels with gum Arabic, food on top of mesh roof
Social	
-Group housing	Visual barriers, animal caretakers
-Single housing	Mirrors; visual, olfactory, auditory and tactile contact with conspecifics; animal caretakers
Other	Cardboard boxes, toys, chews

7. Macro-environment

7.1 Temperature

Animals should be kept within room temperature ranges appropriate for the species. It is important to keep room temperatures relatively constant to avoid difference in research outcomes (i.e. altered food and water consumption (Weihe 1971) as well as the animals' well-being (CCAC 1984; NRC 2011). To maintain body temperatures under any given environmental temperature, animals will adapt on a behavioral (i.e. activity and/or use of resources such as shelter or nesting/bedding material) as well as a physiological level (i.e. metabolism) (NRC 2011). For example, to maintain body temperatures under low room temperatures, mice will huddle, build nests or sleep to control their microclimate (European Directive 2010/63/EU; NRC 2011). It is advised to keep the room temperatures around the minimum or even below the lower temperature range appropriate for the species in order to avoid heat stress. However, this should only be done if the animals are given the appropriate resources (e.g. nesting material or shelter) in order for them to increase their body temperature and avoid cold stress. This provision of resources is also important since the enclosure temperature can be up to 6 degrees higher compared to the room temperature. Especially in groups of rodents which are housed in solid-floored enclosures. Therefore if the temperatures are within the boundaries, rats and mice will have some control on how they manage with different temperatures (e.g. huddling, burrowing or nest building) (Victorian Codes of Practice 2004). Furthermore the design and choice of material will have an influence on the temperature inside the enclosure as metal or wire cages will lose heat more quickly than plastic ones. Another issue arises with the use of racks, the top and middle cages can have an increase 5°C therefore careful monitoring of the temperatures is advised (Victorian Codes of Practice 2004), as well as daily measurement and logging of the room and enclosure temperatures (Council of Europe 2006).

7.2 Ventilation

Adequate ventilation should be provided in the holding room (i.e. the room in which the animal enclosure is located) or macro-environment of the animal, as well as in the enclosure or micro-environment of the animal. Climatic variables such as ventilation are generally set and controlled for the macro-environment except when animals are housed in Individually Ventilated Cages (IVCs) or ventilated rack systems (Victorian Codes of Practice 2004). Proper ventilation should ensure good air quality, provision of fresh air and a stable environment (NRC 2011). Moreover, ventilation is needed to minimize levels and spread of odors, noxious gases, dust and infectious agents of any kind. It also influences humidity and temperatures (e.g. removal of excess heat) (Council of Europe 2006; NRC 2011). Conditions in the animal enclosure are very important when setting and controlling the ventilation for the macro- and micro-environment (Victorian Codes of Practice 2004). Stocking density, size and design of the enclosure are all factors which have to be considered when choosing the proper ventilation settings. For example, differences in ventilation exist between different enclosure floors. Wire grid floors contain approximately 90% of the room ventilation rate while solid floors have 60% (Victorian Codes of Practice 2004). Additionally, the use of filter caps/tops restrict air exchange and may cause an ammonia increase of 50-100% (CCAC 1984; Victorian Codes of Practice 2004). Providing bedding and/or nesting material as well as open or closed shelving, reducing stocking densities or avoiding filter caps can be ways to reduce ammonia levels and influence ventilation rates necessary (Victorian Codes of Practice 2004). With regards to the use of IVCs, location of the air supply in the cage, cage size, ventilation rate and the presence of nesting material influences the well-being of the animal (Baumans et al. 2002). Concentrations of carbon dioxide in IVCs should be less than 5000 ppm (0.5%) and high velocity air should be avoided (Baumans et al. 2002; Krohn et al. 2003; Victorian Codes of Practice 2004). Regardless of type of ventilation system is chosen, noise-disturbance should be avoided and smoking around the animals should be forbidden (Council of Europe 2006). Also, no matter how efficient the ventilation system is it will not compensate for poor cleaning routines together with large increases in relative humidity (CCAC 1984; Victorian Codes of Practice 2004).

7.3 Humidity

Animals should be housed within humidity ranges appropriate for the species as the relative humidity differs in importance depending on the species. Room ventilation and stocking density influences the relative humidity (Victorian Codes of Practice 2004). The relative humidity does not have to be controlled as narrowly as temperature for many mammals (Victorian Codes of Practice 2004; NRC 2011). On the other hand, extreme low or high humidity, especially in combination with extreme temperatures, can cause problems such as an increase in pre-weaning mortality in mice (Clough 1982) or ringtail (i.e. ischemic necrosis of the tail and sometimes toes) in rats (Crippa et al. 2000). In general a humidity range of 30% to 70% is acceptable for most mammalian species (NRC 2011). Keeping the humidity ranges narrow (e.g. 50-55% for rats (CCAC 1984)) can be beneficial to the research as it prevents large fluctuations, in combination with temperature variations, from having an indirect effect on food and water consumption (Weihe 1971). However, together with other parameters such as temperature and ventilation, maintaining a relative humidity within a 5% range will be very difficult and perhaps not always feasible.

7.4 Illumination

Periods of light and dark should be created for the animals to ensure their health and well-being (Dauchy et al. 2011). Where natural light/dark cycles does not occur or is not appropriate to the species, artificially light/dark cycles should be generated by means of controlled lighting to meet and satisfy the biological needs of the animal (European Directive 2010/63/EU). Most commonly used laboratory rodents are nocturnal or crepuscular (Victorian Codes of Practice 2004; NRC 2011). Their eyes are therefore adapted to dim light conditions (Victorian Codes of Practice 2004). Light levels in the enclosure should therefore be low (European Directive 2010/63/EU). Periods of red light frequencies undetectable for rodents can be used to monitor rodents in their active phase (European Directive 2010/63/EU). Albino animals are known to be more sensitive to light (Beaumont 2002) and light-induced retinal damage (i.e. phototoxic retinopathy) can occur even under normal light conditions (i.e. over 60 lux). Blindness can occur at light levels over 100 lux for longer than 16 hours (Victorian Codes of Practice 2004). Therefore, adjusted light levels should be used when housing albino animals. Shaded tops should be present on all racks to prevent retinal degeneration, especially when housing albino animals (European Directive 2010/63/EU). Additionally, shelters or bedding should be provided within the enclosure to enable animals to control their exposure to light and retreat to lower light intensities, especially animals located at the top rack and/or albino animals (Victorian Codes of Practice; NRC 2011).

Light-at-night (LAN) contamination or light pollution should be avoided at all times since it has been shown that a LAN as little as 0.2 lux during a normal light/dark phase suppressed physiologic nighttime melatonin levels. This led to markedly disrupted circadian regulation of physiology and metabolism in nontumour-bearing host animals (Dauchy et al. 1997; 1999; 2011) and stimulated the metabolism and proliferation of human cancer xenografts in rats (Black et al. 2005). In addition, light pollution of 5 lux during a normal light/dark cycle of mice altered their behavior, it altered home-cage locomotor activity and increased anxiety and some depressive responses (Bedrosian et al. 2013).

7.5 Noise

Noise will unavoidably be present in the animal research facilities as animal caretaker activities as well as the animals themselves will produce noise. However, there should be noise control within the facility as extreme and long-term noises can have negative effects on the animals' well-being. For example, exposure to sounds louder than 85dB increased blood-pressure in non-human primates (Peterson et al. 1981). Prolonged noise over 100dB, or 160dB short-term, should be avoided as this causes inner ear damage, noise-induced seizures and other problems to rodents (Victorian Codes of Practice 2004). Furthermore, laboratory animals such as rodents are sensitive to ultrasound (Oliver et al. 1994) and many laboratory devices produce such a sound like dripping taps, trolley wheels and computer monitors. This may cause abnormal behavior, disrupted breeding cycles and can become an uncontrolled variable for research experiments (Council of Europe 2006; NRC 2011). Rodents can hear frequencies which are inaudible to humans as rodents communicate at 10-70 kHz whereas the human audible range is up to 20 kHz (Victorian Codes of Practice 2004). It is therefore advisable to monitor the acoustic environment on different frequencies over long periods (Council of Europe 2006). Furthermore, separation of human and animal areas as well as loud, noisy animals from the quieter ones will reduce noise disturbances (NRC 2011). The use of radio as background noise for rodents is not advisable due to the contradicting results (Baumans 2010b; Krohn et al. 2011). With regards to NHPs it could be a useful enrichment and reduce the startle reflex of primates (Jennings and Prescott 2009). However, when providing radio or music to NHPs, careful monitoring of changes in behavior should be carried out as there is not enough data or information about the short- and long-term effects of radio on the welfare of the animals.

8. Mice

8.1 Temperature

Mice should be housed within temperature ranges of 18°C to 26°C, see Table 9. Slight differences exist between guidelines and resources where temperature requirements range from 18°C-24°C (Victorian Codes of Practice 2004), 20°C -24°C (Council of Europe 2006) and 20°C -26°C (NRC 2011) and the minimum and maximum temperatures are outlined in Table 4. Depending on design, choice of material and stocking density, temperatures in these ranges together with provision of resources (e.g., provision of shelters or nesting material) will be satisfactory. Exception from these temperature ranges should be made with regards to very old, very young or hairless animals where the room temperature may have to be higher than the requirements advised. Also, suitable bedding and/or thermal pads should be provided to these animals (Victorian Codes of Practice 2004).

8.2 Ventilation

The appropriate ventilation rate for mice depends on the stocking density, size and/or provision of substrates. For example, under normal stocking densities ten to twenty air changes per hour for conventional holding rooms will suffice. However, if stocking densities are low, eight to ten air changes per hour will suffice (Victorian Codes of Practice 2004; Council of Europe 2006). Also if filter tops are used than a higher ventilation rate may be necessary. Average concentrations of ammonia should not exceed 25ppm in both the micro- as the macro-environment (Victorian Codes of Practice 2004). With regards to IVC, high intra-cage ventilation rate can induce chronic stress and heat loss by draught (Baumans et al. 2002; Krohn et al. 2003). Air speed rates should be kept below <0.2 m/s (Lipman 1999). The rate of air changes per hour should be kept below 80, provision of nesting material is beneficial and the air inlet on top should be preferred (Baumans et al. 2002).

8.3 Illumination

Lighting intensity should be below 325-350 lux in the room where the enclosure is located at one meter height. (Victorian Codes of Practice 2004; NRC 2011). When housing albino mice, light intensity levels under 100 lux should be maintained (Victorian Codes of Practice 2004). Moreover, rats and mice prefer lower light intensity levels such as 40 lux (Blom et al. 1996). A daily cycle of 12 hours light and 12 hours dark is most commonly used and satisfactory.

8.4 Noise

It is advisable to keep background noise (i.e. long-term noise) under 50 dB, this includes ultrasound. Regarding short-term noise, this should be kept under 85dB (CCAC 2003; Victorian Codes of Practice 2004; NRC 2011).

8.5 Humidity

A relative humidity of 40% to 70% is recommended for housing mice (Victorian Codes of Practice 2004). Other resources have narrowed it down to 45-65% (Council of Europe 2006) or 50-70% (CCAC 2003).

9. Rats

9.1 Temperature

Rats housed within temperature ranges of 18°C to 26°C should be satisfactory. Slight differences exist between guidelines and resources where temperature requirements range from 18°C-24°C (Victorian Codes of Practice 2004), 20°C -24°C (Council of Europe 2006) and 20°C -26°C (NRC 2011). Depending on design, choice of material and stocking density, temperatures in these ranges together with provision of resources will be satisfactory. Exception from these temperature ranges should be made with regards to very old, very young or hairless animals where the room temperature may have to be higher than the requirements advised. Also, suitable bedding and/or thermal pads should be provided to these animals (Victorian Codes of Practice 2004).

9.2 Ventilation

Similar to mice, the appropriate ventilation rate for rats depends on the stocking density, size and/or provision of substrates. Ten to twenty air changes per hour for conventional holding rooms will suffice under normal stocking densities. However, if stocking densities are low, eight to ten air changes per hour will suffice (Codes of Practice 1997; Council of Europe 2006). Also if filter tops are used than a higher ventilation rate may be necessary. Average concentrations of ammonia should not exceed 25ppm in both the micro- as the macro-environment (Victorian Codes of Practice 2004). With regards to IVC, high intra-cage ventilation rate (i.e. 80 – 120 air changes per hour) can induce chronic stress and heat loss by draught (Baumans et al. 2002; Krohn et al. 2003). Air speed rates should be kept below <0.2 m/s (Lipman 1999). The rate of air changes per hour should be kept below 80, as this was preferred by rats (Krohn et al. 2003), but maintained at a minimum of 30-40 changes per hour to prevent concentrations of NH_3 in the cage (Reeb et al.1998, Reeb-Whitaker et al.2001).

9.3 Illumination

Lighting intensity should be below 325-350 lux in the room where the enclosure is located at one meter height. (Victorian Codes of Practice 2004; NRC 2011). When housing albino rats, light intensity levels under 100 lux should be maintained (Victorian Codes of Practice 2004). Moreover, rats and mice prefer lower light intensity levels such as 40 lux (Blom et al. 1996). A daily cycle of 12 hours light and 12 hours dark is most commonly used and satisfactory.

9.4 Noise

It is advisable to keep background noise (i.e. long-term noise) under 50 dB, this includes ultrasound. Regarding short-term noise, this should be kept under 85dB (CCAC 2003; Victorian Codes of Practice 2004; NRC 2011).

9.5 Humidity

A relative humidity of 40% to 70% is recommended for housing rats (Victorian Codes of Practice 2004). Other resources have narrowed it down to 45-65% (Council of Europe 2006) or 50-55% (CCAC 1984; 2003). However, together with other parameters such as temperature and ventilation, maintaining a relative humidity within a 5% range will be very difficult, therefore maintaining humidity levels within larger ranges such as 40%-70% is more feasible.

10. Guinea pigs

10.1 Temperature

Guinea pigs should be housed within temperature ranges of 18°C to 26°C. Slight differences exist between guidelines and resources where temperature requirements range from 18°C-24°C (ARRP 2006), 20°C -24°C (Council of Europe 2006) and 20°C -26°C (NRC 2011). Guinea pigs are able to better withstand cold than heat, if provided with sufficient bedding and protection from draughts (Victorian Codes of Practice 2004; ARRP 2006). If room temperatures are above 25°C than reproductive rates will decline. Depending on design, choice of material and stocking density, temperatures in these ranges together with provision of resources (e.g. provision of shelters or hay) will be satisfactory. Exception from these temperature ranges should be made with regards to very old, very young or hairless animals where the room temperature may have to be higher than the requirements advised. Also, suitable bedding and/or thermal pads should be provided to these animals (ARRP 2006).

10.2 Ventilation

Similar to mice and rats, the appropriate ventilation rate for guinea pigs depends on the stocking density, size and/or provision of substrates. For example, ten to twenty air changes per hour for conventional holding rooms will suffice under normal stocking densities. However, if stocking densities are low, eight to ten air changes per hour will suffice (Council of Europe 2006; ARRP 2006). Also if filter tops are used than a higher ventilation rate may be necessary. Average concentrations of ammonia should not exceed 25ppm in both the micro- as the macro-environment (Victorian Codes of Practice 2004). Furthermore, it has been suggested with regards to guinea pigs that the average ammonia concentration should not exceed 10 ppm (Seltzer et al. 1969; Animal Resources Centre 2005; ARRP 2006). With regards to IVC, high intra-cage ventilation rate can induce chronic stress and heat loss by draught (Baumans et al. 2002; Krohn et al. 2003). Air speed rates should be kept below <0.2 m/s (Lipman 1999). The rate of air changes per hour should be kept below 80, provision of nesting material is beneficial and the air inlet on top should be preferred (Baumans et al. 2002; Krohn et al. 2003).

10.3 Illumination

Lighting intensity should be below 325-350 lux in the room where the enclosure is located at one meter height. (Victorian Codes of Practice 2004; NRC 2011). When housing albino animals, light intensity levels under 100 lux should be maintained (Victorian Codes of Practice 2004). Furthermore a daily cycle of 12 hours light and 12 hours dark is most commonly used and satisfactory. Although a daily cycle of 16 hours light and 8 hours dark can be used as well (North 1999; ARRP 2006).

10.4 Noise

It is advisable to keep background noise (i.e. long-term noise) under 50 dB, this includes ultrasound. Regarding short-term noise, this should be kept under 85dB (CCAC 1984; Victorian Code of Practice 1997; NRC 2011). Extra care and attention should be taken regarding guinea pigs as they startle and panic easily which could lead to injuries (Victorian Code of Practice 1997; Kaiser et al. 2010). It is therefore suggested to have some sort of background noise to diminish the panic reaction at sudden loud noises. A background noise of 50dB has been suggested to avoid disturbance to animals and personal (Clough 1999; ARRP 2006).

10.5 Humidity

A relative humidity of 40% to 70% is recommended for housing guinea pigs (Anderson 1987; ARRP 2006). Other resources have narrowed it down to 45-65% (Council of Europe 2006) or 50-60% (CCAC 1984; Kaiser et al. 2010).

Table 9. Macro-environment requirements regarding mice, rats and guinea pigs. Values are based on Canadian Council of Animal Care (1984; 2003); Council of Europe (2006); Animal Research Review Panel 2006; Victorian Codes of Practice 2004 and The National Research Council (2011).

Species	Temperature (°C)	Ventilation (air changes per hour)	Lighting (lux)*	Noise (short-term) (dB)	Humidity (%)
Mice	18°C -26°C	8-20	<350	<85dB	30%-70%
Rats	18°C -26°C	10-20	<350	<85dB	30%-70%
Guinea pigs	18°C -26°C	4-20	<350	<85dB	30%-70%

* when housing albino rodents light intensity levels should be < 100 lux

11. Non-human primates

11.1 Temperature

Non-human primates should be housed within the appropriate temperature ranges. If the primates have access to an outdoor enclosure, shelter from various weather conditions should be available as well as continuous access to heated indoor enclosure (Council of Europe 2006). The ideal room temperature will vary with different primate species (CCAC 1984). Very broad ranges include 18°C to 29°C for housing non-human primates (NRC 2011). However, more species-specific ranges have been determined, see Table 10. Tropical New World primates (i.e. marmosets and tamarins may require higher temperature ranges such as 22°C-28°C (CCAC 2003; Council of Europe2006). As for squirrel monkeys, temperatures ranging from 22°C-26°C is more suitable. If the animals are restricted in their movement, for experimental reasons, room temperatures around 26°C are advised (Council of Europe2006). Temperature ranges from 16°C-25°C are satisfactory for rhesus monkeys, vervets and stump-tailed macaques. As for long-tailed macaques, ranges from 21°C - 28°C are suitable (CCAC 2003; Wolfensohn and Honess 2005d; Council of Europe 2006). Baboons require temperatures between 16°C-28°C (CCAC 1984; Council of Europe 2006).

11.2 Ventilation

Adequate ventilation should be present in the NHP enclosure room to provide fresh air, good quality and a stable environment (NRC 2011). Under low stocking densities 8 to 10 air changes per hour will suffice (Council of Europe 2006). Under normal stocking densities, a minimum of 10 to 20 air changes per hour is required (CCAC 1984; Council of Europe 2006; NRC 2011). However under high stocking densities, 18 to 20 air changes per hour are needed (CCAC 1984).

11.3 Illumination

Where possible, natural lighting (e.g. skylights and/or windows) is encouraged (NHMRC 2003; NC3Rs 2006). If this is not available, controlled lighting should be present. A 12 hour light/12 hour dark cycle is satisfactory when housing NHPs. Dawn/dusk control is preferred as sudden switches to light or dark may startle the animals and could cause injuries as they may jump at the time the lights are switched off (Jennings and Prescott 2009). For some nocturnal species (i.e. who will be active during the night), partial usage of dim red light during the working day allowing the animals to be observed during their active periods and routine husbandry activities to be carried out is advisable (Council of Europe 2006). Regarding squirrel monkeys, the light provided should resemble the light spectrum of daylight. The amount of daylight provided should not be less than eight hours. Also, providing limited exposure to an UV-lamp could enable synthesis of Vitamin D3 (Council of Europe 2006). As for marmosets, higher light intensities which also produce heat should be considered as they have better reproduction and show ‘sunbathing behavior’ (Heger et al. 1986).

11.4 Noise

The minimum level of background noise should be kept below 65 dB for long periods of time (Council of Europe 2006). However, effort should be made to keep background noise under 50dB (Clough 1999). Background noise such as music, radio programs or nature sounds can be used as a type of enrichment as it disguises sudden loud noises or unfamiliar sounds (Council of Europe 2006; Baumans et al. 2013). However, the radio or music should not be louder than human conversation or used continuously (Jennings and Prescott 2009). Furthermore, some species, such as marmosets, are sensitive to ultrasound thus measures should be taken to avoid laboratory devices causing ultrasound noise disturbance.

11.5 Humidity

In general, NHPs housed within humidity ranges from 40%-70% should be satisfactory (CCAC 1984; Council of Europe 2006). Some primate species living in tropical rainforests will have higher humidity levels in nature. Effort should be made to keep the relative humidity levels stable as fluctuations are less well tolerated by marmosets and tamarins. A smaller relative humidity range (i.e. 40% - 60%) for these species is therefore advised (Poole et al. 1999). Furthermore, humidity levels should not drop below 30% for longer periods of time, especially for tropical New World species as respiratory problems may arise (CCAC 1984; Council of Europe 2006; Rennie and Buchanan-Smith 2006).

Table 10. Macro-environment requirements regarding non-human primates. Values are based on the following documents: Canadian Council of Animal Care 1984; 2003; Council of Europe (2006); National Research Council (2011).

Species	Temperature (°C) Room cage	Temperature (°C) Pen Free Ranging	Ventilation (changes per hour)	Lighting (light/ dark)	Noise (short -term)	Humidity (%)
Baboons	16 C°- 28 C°	15 C° - 30 C°	10-20	12-12	<65dB	40% - 70%
Rhesus monkeys, stump-tailed macaques and vervets	16 C°- 25 C°	18 C° - 29 C°				40% - 70%
Long-tailed macaques	21 C°- 28 C°	18 C° - 29 C°				40% - 70%
Squirrel monkeys	22 C°- 26 C°					40% - 70%
Marmosets and tamarins	22 C°- 28 C°					40% - 60%

12. Discussion

Housing non-human primates is generally considered more difficult than other species such as rats or mice. The close phylogenetic relationship with humans, high level of curiosity, low boredom threshold and high sensitivity to disturbance makes it very difficult to meet all the environmental, behavioral, psychological and physical needs when housing a non-human primate (Wolfensohn and Honess 2005a). Providing a large complex social environment (e.g. appropriate for rhesus macaques) or adequate amount of space are constrains when designing appropriate, economically and ergonomically beneficial housing facilities while also preventing the development of abnormal behavior and poor welfare (Novak et al. 2013). In order to create an optimal housing environment by means of providing enrichment and/or social interactions, which are discussed previously, establishing a good relationship between caretaker/staff and the animal may also enrich the lives of NHPs and improve their well-being, especially when NHPs are individually housed (Wolfensohn and Honess 2005c). Even though it has long been thought that a close bond between NHPs and their caretaker ought to be avoided as this can undermine the objectivity of the research, thoughts and opinions are starting to change (Vitale and Pollo 2011). Positive social interaction with humans can be rewarding for both the primate and the caretaker as this can significantly reduce stress levels when routine procedures have to be carried out (e.g. blood samples or cleaning) and improve the NHP well-being (Reinhardt 1997; Wolfensohn and Honess 2005c; Coleman 2011). An example of this was shown when the care of two primate facilities were compared. At the first facility, the primates were considered ‘objects’ and staff often used intimidation and physical strength when certain actions were demanded from the animals. At the second facility the primates were treated with respect and the staff had empathy for them. The primates of the second facility were healthier and had better well-being than primates of the first facility (Arluke & Sanders, 1996; Coleman 2011). Additionally, positive relationships between staff and NHPs can reduce abnormal behavior and increase species appropriate behavior (e.g. grooming) (Bayne et al. 1993; 2002; Reinhardt 1997; Baker 2004; Manciocco et al. 2009; Coleman 2011). Positive social interactions between caretakers and NHPs can be obtained by providing enrichment (Bayne et al. 1993), positive reinforcement training or play (Baker 2004; Manciocco et al. 2009).

When it comes to training of NHPs, positive reinforcement training (PRT) should be used at all times when animals have to cooperate with research and husbandry procedures (e.g. injections or cleaning routines) as this can reduce stress and fear of these common management procedures (Bassett et al. 2003; Wolfensohn and Honess 2005c; Coleman 2011). Furthermore, restraint by pharmacological means can be reduced by using PRT (Coleman 2011). The PRT technique (i.e. where a positive reward follows when the desired behavior is performed by the NHP (Pryor 1999) can also be used to positively reinforce the development of a positive relationship between caretaker and NHP (Wolfensohn and Honess 2005c). Using fruit as a reward is beneficial as this will not interfere with the nutritional requirements of the diet of the research as fruit is composed 80% out of water (Wolfensohn and Honess 2005c). Also, PRT can be beneficial to the caretaker as the animal may associate the presence of a caretaker with positive experiences such as food rewards or cognitive activities. The animals may then show affiliative rather than aggressive behavior towards the staff and learn to cooperate in future activities (Wolfensohn and Honess 2005c). Therefore, PRT is a significant way of refinement and should be used when possible when working with NHPs.

Developing a close bond between staff and the NHPs can also have negative consequences. Caretakers can develop very strong attachments and preferences towards certain animals and favor them. If this happens than caretakers must be extra cautious in dividing their attention, time, toys and food treats equally and not favor certain primates as this can influence scientific outcomes. Also, problems or injuries of other animals may be unintentionally neglected this way (Russow 2002; Coleman 2011). Basically, staff can make emotional and empathic decisions (i.e. which is reinforced by developing a close relationship with NHPs) which may not be scientific protocol (e.g. providing food treats while this is prohibited due to experimental conditions). Facilities can avoid this problem by teaching and training the staff how to properly interact with NHPs and for example have meetings with all the staff by which these kinds of incidents are discussed. Additionally, explaining the research protocol and significance of changes of care can reduce the likelihood of staff breaking protocol (Coleman 2011). Next to favoritism, challenges may arise on an emotional level where the caretaker may find it harder to cooperate in the execution of painful or stressful procedures. Again, explaining the significance of the research or being part of a committee (e.g. the Institutional Animal Care and Use Committee) where they can express their ethical concerns may aid with this problem (Coleman 2011).
Despite the possible negative consequences, developing a close relationship and positive social interactions with NHPs may also increase moral and job satisfaction which in turn leads to better care and improved animal well-being (Waitt et al. 2002; Coleman 2011).

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