COMFORTABLE QUARTERS FOR LABORATORY ANIMALS

edited by Cathy Liss, Kenneth Litwak, Dave Tilford, and Viktor Reinhardt

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Animal Welfare Institute

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TENTH EDITION

The Animal Welfare Institute is a nonprofit charitable organization founded in the United States in 1951 and dedicated to reducing animal suffering caused by people. AWI engages policymakers, scientists, industry, and the public to achieve better treatment of animals everywhere—in the laboratory, on the farm, in commerce, at home, and in the wild.

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Cover photograph by Dr. Brianna Gaskill

This book is devoted to those who recognize the need to improve the welfare of animals in research and have the will to make it happen.

Foreword

More than 30 years ago, I began conducting visits at registered research facilities for the Animal Welfare Institute (AWI). I observed a wide range of different species and their housing, handling and care, along with surgical sites, storage rooms, offices, and libraries. I also spent time meeting with various staff at research laboratories across the United States. Most laboratories appeared familiar with AWI's reputation and were willing to open their doors to me, if only a little. While I was not always allowed to see all of the animals or facilities, the interactions I observed and had with the technicians, investigators, and management staff told me much about their animal care programs. Perhaps most telling was the interaction between the people and the animals. How did staff members behave as they entered an animal room and how did the animals respond?

What was nearly universal in the facilities I visited for many years was that the animals were kept in small, barren cages with feeders and waterers—and nothing more. Enrichment was unthinkable, uncharted territory, viewed by labs as both costly and a source of extraneous variables that would threaten research results. Nonhuman primates (with the exception of breeding animals), dogs, and other social species were individually housed. I routinely witnessed primates engaging in a range of stereotypies, including hair plucking and self-mutilating. I saw dogs cowering and shaking in the backs of their tiered cages, while others were circling round and round in their small confines. I observed rabbits sitting in the middle of their cages, not moving because there was no room or reason to do so. I saw many rodents being kept in wire-bottom cages, while those in shoebox cages only had a bit of litter on the floor.

Now, it appears that a reversal in perspective is underway. There is increasing recognition of the need to keep animals physically and psychologically healthy to *reduce* extraneous variables—and this is done by providing them with species-appropriate housing and enrichment and reducing pain and distress when possible. The eighth edition of the *Guide for the Care and Use of Laboratory Animals*, published in 2011, embraces this view (albeit without the regulatory force of the Animal Welfare Act). Similarly, the Institute of Medicine and the Working Group of the Council of Councils (an advisory body to the NIH) recommend significant improvements in the manner in which chimpanzees are housed, with the Working Group calling for "ethologically appropriate physical and social environments." This recommendation was later accepted in large part by the NIH.

Spending significant moneys on shiny new cages and commercially available enrichment devices may not be necessary, though; simple steps such as reconfiguring old cages and making your own enrichment can have a positive impact on animal welfare, while also minimizing research confounds. Teaching animals to cooperate with positive reinforcement instead of forcing them into compliance is increasingly recognized as beneficial to the animals as well as to research outcomes.

Agreeing with the change in perspective is one thing, but implementing it for all animals is quite another. While the situation for animals in research is changing, and improvements have been made, there is still much that needs to be done. The vast majority of animals used in research—rats, mice and fish—are not covered under the Act, nor are birds, amphibians and other cold-blooded species. The requirements under the Act sorely need to be updated and expanded. All animals deserve an environment adequate to promote their well-being.

We at AWI hope you will take inspiration from this book to go well beyond the minimum standards in seeking to ensure the best possible welfare of the animals who are completely dependent upon you. This book is intended for anyone involved with animals in laboratories—technicians, veterinarians, scientists, institutional officials, enrichment specialists, IACUC members, and inspectors. Thank you to those who are already moving the bar ever higher. To those who aren't there yet, we don't underestimate the task before you in trying to facilitate change, but such change is warranted, and we hope this book will be helpful to you.

It has been 13 years since the previous edition of this book was published and this new edition is more than twice the length, in part because research on improved housing and enrichment has been, and continues to be, conducted. There are 14 chapters on different animals in research (including new chapters on ferrets and zebrafish, as well as chapters on extraneous variables and the human-animal bond) that describe species-specific needs and offer recommendations on how to address them in the laboratory.

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In the previous edition of *Comfortable Quarters,* the chapter on housing mice focused on what was known from wild mice and how we can take that into account when providing them with optimal housing conditions. Since our knowledge of wild mice is the basis for our understanding the needs of laboratory mice, these issues will certainly be addressed again in the majority of paragraphs of this sequel, supplemented with information from more recent literature. Our understanding of the needs of mice is not only a prerequisite when designing comfortable quarters, but also when performing procedures with minimal stress. Thus, in the last paragraphs we will address several procedures that are commonly performed on laboratory mice and provide the reader with tips and tricks as to how these procedures can be performed in a way that is least stressful for both the animal and the caretaker.

Mice in the wild

The house mouse, *Mus musculus*, is one of the most common mammals on earth. This animal is believed to have originated in Central Asia and subsequently dispersed widely around the globe, where it displays remarkable variations in color and size as an environmental adaptation (Marshall, 1978). There is evidence that the house mouse has lived in close proximity with humans since the end of the last glacial period (about 12,000 years ago; Hedrich, 2012, chapter 1). Mice inhabit most parts of the world where humans live. Perhaps because mice live in such close proximity with humans and are so successful in outsmarting people when it comes to their survival, the house mouse has often been the subject of study. Specifically advisable readings are "Mice all over" (Crowcroft, 1973) and the chapter on the laboratory mouse in the *UFAW Handbook*, which includes an elaborate table on mouse standard biological parameters (Baumans, 2010). In the next paragraphs we will provide a brief

outline of house mouse biology and behavior to increase the reader's understanding of the precarious balance between their extraordinary adaptability to extreme circumstances and the boundaries thereof in captivity.

Life cycle

Typically, wild female mice begin breeding at 6–7 weeks of age, and have their first litter around the age of 9–10 weeks, after a 20-day gestation (on average). Newborn mouse pups are born hairless (with the exception of whiskers), deaf, and blind, and are completely dependent on their mother for survival. Over the course of 2 to 3 weeks, they subsequently start growing fur, their incisors erupt, their ears open, and finally their eyes open (Baumans, 2010). These life events happen in a strict order at set times, as does the onset of their motor skills, starting as rooting and circling in the first week and presenting as refined activities and sensory responses in the third week, before weaning (Fox, 1965). Around 3 weeks of age, the pups start to explore the immediate surroundings of their nest and increasingly become more independent of their mother for food (Latham & Mason, 2004). This strictly ordered development is very useful to detect any developmental effects in laboratory mice and is used on a wide scale as part of phenotyping the large number of transgenic mouse lines (Van der Meer, 2001).

The young mice grow up in demes (territories populated by family groups), usually consisting of a dominant male, several females with their progeny, and subordinate males. Female juveniles may stay or disperse as adults to neighboring demes. Unfamiliar males are chased out of the territory while juvenile and subordinate adult males are, to a certain extent, tolerated by the dominant male within the boundaries of the deme. Evidence suggests that the level of tolerance depends on population numbers and food availability, with highest tolerance in densely populated areas with an abundance of food (Crowcroft, 1973; Mackintosh, 1970; Mackintosh, 1973; Poole & Morgan, 1976; Hurst et al., 1993). When population density increases further, subordinate (juvenile) males may disperse out of the territory and live in bachelor groups (Busser et al., 1974).

Wild mice have an average life span of 9–18 months, although some can live up to 2 or even 3 years in captivity or protected environments. The oldest mouse ever to have lived, as far as we know, was kept in captivity in an enriched environment, but did not receive any genetic, pharmacological, or dietary treatment and lived for 1,551 days (about 50 months; Than, 2006). Usually, wild mice live less than a year, due to the high level of predation, pest control, and exposure to harsh living conditions.

Senses and behavior

In the wild, mice are most active from dawn to dusk and then seek shelter from bright light during the daytime. Thus, it is not surprising that mice have excellent senses of smell, taste, touch, and hearing, but have generally poor vision. However, they do have good peripheral vision that allows them to detect movement.

The mouse retina consists of rods and two varieties of cones, one serving the traditional green-yellow region of the vision spectrum and another serving the ultra-violet (UV) region, essentially invisible to humans and many other mammals. These UV cones are more concentrated in the ventral retina, which may reflect the background spectrum of the sky at times of the day or twilight when mice are most active. UV sensitivity and the presence of two varieties of cones (UV and green-yellow) that are wide apart in the spectrum increase contrast, and thus help mice distinguish form and movement achromatically (Gouras & Ekesten, 2004).

The mouse auditory and vocalization apparatus has evolved to hearing and emitting frequencies well beyond the human auditory range. Their hearing is especially sensitive to sounds in the 5–20 kHz range and around 50 kHz (Baumans, 2010), which is beyond the human auditory range (20 Hz to 20 kHz). Both audible and ultrasonic calls are used for communication. Pups emit wriggling calls (<10 kHz) to invoke nursing behavior of the mother and distress calls (50–70 kHz) when separated from their mothers (Latham & Mason, 2004). Male mice grunt and squeak audibly (1–2 kHz) during aggressive encounters and sing ultrasonic courtship songs to females. Female mice can discriminate between the characteristics of male songs and prefer the songs of males of different strains. It is probable that male songs contribute to kin recognition by females, thus avoiding inbreeding and resulting in greater heterozygosity of offspring (Kikusui & Koide, 2011).

Not only the auditory and vocal apparatus, but also the olfactory sense plays an important role in social communication between wild mice. Pheromones from urinary and plantar glands are used to mark territories and to recognize which individuals are familiar. They may invoke or suppress aggression between males and influence mating behavior (Hurst et al., 1993; Hurst et al., 1998; Humphries et al., 1999; Nevison et al., 2000). Mice use their olfactory sense to receive information on edible foods by smelling the breath of other mice (Munger et al., 2010) and use social cues in choosing feeding sites (Baker, 1985). Mice nibble whatever food is available, eating small portions during many feeding bouts throughout both day and night (Baumans, 2010). These olfactory and behavioral strategies are an excellent way to find out which novel foods are edible and which are not. Mice are omnivorous and are able to chew through almost everything. Their diet, typically 10–15% of their body weight per diem, typically consists of seeds and grains, but they also eat roots, leaves and stems, and insects such as beetle larvae, caterpillars, and cockroaches. Mice, like other rodents, also engage in coprophagy, typically consuming up to 10% of fecal matter as a means of nutritional supplementation (Heinrichs, 2001).

The mouse is a very agile creature. When not eating, mice spend their time exploring their surroundings and engage in running, climbing, digging, and even swimming when they need to. Their ability to climb vertical walls, jump as high as 30 cm and squeeze through cracks as small as 0.5 cm allows them to reach almost any area. Since mice have generally poor eyesight, they explore their surroundings by moving along barriers, touching the walls with their whiskers and sides of their bodies. Although they generally avoid open spaces (thigmotaxis), they are curious and explore their surroundings continuously, memorizing pathways, obstacles, food, water, shelter, and other elements in their habitats.

Mice sleep in several short and long bouts throughout the day, with the longer sleeping bouts typically occurring during the daylight phase (Van de Weerd et al., 1997). Each sleeping bout is preceded by an elaborate amount of nesting behavior. The nests,

DOME SHAPE NEST, BUILT BY A BALB/C MOUSE

CUP SHAPE NEST, BUILT BY A C57BL/6 MOUSE

equally formed by female and male mice, are constructed of any soft material the mice can shred and form to their liking. Mice originating from surface nesters (e.g., commensals of some field mice, as well as the laboratory strain BALB/c) typically build dome-shape nests with single tiny openings. Burrow nesters such as C57BL/6, on the other hand, build more cup-shape nests. This difference in nest-building behavior is believed to be genetically determined and can still be found in laboratory strains today (Van de Weerd et al., 1997; Sluyter & Van Oortmerssen, 2000). Nest building behavior is an excellent way to check the health of mice (Deacon, 2012; Jirkof et al., 2013). Mice who are subclinically ill build increasingly frumpy nests or fail to build a nest at all. This behavior has been successfully applied in several mouse strains to establish subclinical disease (Jirkof et al., 2010; Deacon, 2012).

Essentially, wild mice spend their days (or nights) eating, sleeping, reproducing, and exploring—sensing the world in a way considerably different from humans.

Emergence of the laboratory mouse

The question as to why the house mouse was initially chosen (and remains) the most popular laboratory animal is not difficult to answer; it is due to their success in the wild. They are small, immensely adaptive in almost all circumstances, and fast breeders. Above that, they are mammals, sharing about 90% of their genetic makeup with humans (Shakespeare, 2013). In the early 19th century, people—fascinated by this agile little creature with such bewildering polymorphism—started to breed them as pets with specific characteristics such as coat color, and mice from around the world were exchanged to create new lines. Since experimental science started to develop in the late 19th century, the use of these specifically bred pet mice was a logical step, and many of the strains of mice that were bred then are still used today; the most well-known example being the strain C57BL/6, established in the United States by Lathrop as an intercross between the black progeny of female 57. Genetic analysis of most common laboratory mice reveals that they all originate from an intercross between three subspecies of wild mice: *Mus musculus domesticus*, *M. m. musculus* and *M. m. castaneus*. An extensive discussion of the emergence of laboratory mice can be found in Part 1 of *The Laboratory Mouse* (Hedrich, 2012).

Meshing human and mouse needs

With the choice of mice as laboratory animals, came the question how best to house and care for them. The number of laboratory animals has rapidly increased since the mid-20th century, posing constraints on economically feasible housing. At the same time, there has been much attention paid to the importance of standardization to reduce intra- and inter-experimental variability and increase reproducibility of results within and between laboratories (Olsson et al., 2003). This has led to such an intertwining of economically feasible housing and standardization, that the two are routinely viewed as equal, even as the definition of standard housing has evolved. There has been a gradual change from a jar containing some sawdust, to a shoebox-shaped cage with a wire mesh bottom for individual mice, to a shoebox-shaped open cage with sawdust, to the technically ingenious present-day individually ventilated cage (IVC) systems.

Nevertheless, it has been shown that despite rigorous efforts to equalize conditions, inbred mouse strains, which originated simultaneously from three well-recommended laboratories, have significant site-based effects for nearly all variables examined (Crabbe et al., 1999; Van de Weerd et al., 2002; Wahlsten et al., 2003). Further, increased complexity of housing conditions does not necessarily increase variation between animals. It may even be argued that, since it is variation between the animals that we wish to decrease, housing conditions should be designed with respect to individual needs of animals, much like we nowadays treat human beings through personalized health care (Snyderman, 2012).

Boundaries to adaptability

Captivity changes behavior of animals. Animals who have been domesticated generally become more tame, possibly through artificial selection for traits such as ease of handling and decreased aggression. Evidence for this has been clearly demonstrated in pets, zoo and farm animals, and laboratory animals (Jones et al., 2011). These changes in behavior enable animals to better cope with the circumstances. Nevertheless, there is compelling evidence that feral animals (once domesticated and released back into the wild) are still able to exhibit the behaviors of their wild counterparts that are necessary for survival. In general, changes in behavior brought about by domestication are quantitative rather than qualitative (Howard et al., 2010). Berdoy (2003) provided what is perhaps the most illustrative and enjoyable example of this wild behavior that is still present in laboratory animals. Berdoy created a semi-natural farm environment into which he released laboratory rats from two strains: Wistar and Lister hooded rats; here, they had to compete, like their wild cousins, for food, shelter and mates for a period of 6 months. The resulting film, *The Laboratory Rat: A Natural History*, reviews the range of behaviors and needs that, despite generations of domestication, remain innate and ready to be expressed when given the opportunity. The film is highly recommended for both students and teachers in the field of ethology and laboratory animal science. Although a similar experiment has not been performed with mice, it is likely that adaptation of laboratory mice in feral conditions would be similar.

These examples pose the question as to what the boundaries are to adaptability of animals in captivity, especially with regard to behaviors that animals are motivated to perform per se (behavioral needs), even if the physiological need that the behavior serves is fulfilled (see, e.g., Jensen & Toates, 1993).

For laboratory mice, despite their extraordinary capabilities to adapt to changing circumstances, evidence is accumulating that the present-day standardized housing and management is still a long way from an environment meeting their behavioral needs, leading to behavioral problems indicative of decreased welfare—such as stereotypies, aggression, and whisker and fur trimming. The underlying mechanisms for development of these detrimental behavioral patterns are only partly understood and some strains of mice appear to be more prone to developing them than others. There is, however, general consensus that rearing mice in barren, restricted cages, lacking appropriate stimuli, is a precursor for the development of abnormal behavior (Latham & Mason, 2004; Würbel et al., 1996). Garner et al. (2004) found that cage height in the animal room and cage material were factors influencing severity of barbering and stereotypic behaviors, while Nevison (1999) linked them to repetitive and futile attempts to flee from the cage. Aggression, especially between male mice, may be the result of inbreeding, environmentally disturbed social behavior, frustration, or lack of control (Van Loo et al., 2003).

ABNORMAL BEHAVIOR: WHISKER TRIMMING

There is an increasing awareness of the importance of an environment meeting the mice's needs both for animal welfare and for the quality of research, clearly articulated by Poole (1997): "Happy animals make good science." This awareness is evident in what is considered standard today: shoebox-shaped cages with bedding material, nesting material or nest boxes, gnawing blocks, and social housing. Environmental refinement is an ongoing process and we should aim to provide stimuli beyond the satisfaction of the basic needs normally accommodated in standard housing conditions (Baumans & Van Loo, 2013). The notion of catering to behavioral needs has been embedded in present legislation and guidelines around the world, all with more or less similar import: that animals should be (a) allowed adequate space to express a wide behavioral repertoire, (b) socially housed wherever possible, and (c) provided with an adequately complex environment within the animal enclosure to enable them to carry out a range of normal behaviors (see, e.g., European Parliament and Council of the European Union, 2010; National Research Council [of the United States], 2011; National Health and Medical Research Council [of Australia], 2013).

Making use of our knowledge of how mice perceive the world

With proper management of the wide range of stimuli in the laboratory mouse environment—both physically (such as climate, cage furniture and procedures) and socially (other mice, human caretakers)—and through knowledge of the mice's behavior in the wild and knowledge of and empathy for the way laboratory mice experience the world around them, a lot can be achieved, sometimes with only minor adaptations (Van de Weerd & Baumans, 1995).

In the following paragraphs, both scientific and anecdotal evidence on welfare-enhancing adaptations in housing, management and experimental procedures commonly performed on mice are discussed in relation to what we know of their wild counterparts.

Housing and husbandry

Temperature: The climate in animal rooms is usually kept as stable as possible, with temperatures between 20–24°C (68–75°F). Several studies have shown that mice prefer temperatures quite above the temperatures usually provided (Blom et al., 1993; Gaskill et al., 2009). This does not mean that room temperature needs to be increased to levels uncomfortable to work in for animal caretakers. Mice prove to be perfectly able to adapt their microclimate to their needs. Housing the mice socially and providing them with nesting material enables them to create nests with temperatures around 30–32°C (86–90°F; Gaskill et al., 2011; Gaskill, 2013). Under special circumstances, however, it is advisable to increase cage temperature—for example, when mice are housed individually in metabolic cages or when they are recovering from general anesthesia. Again, in these circumstances, it is advisable to give the mice a choice. When providing heating mats after surgery, placing half of the cages on the mat ensures that mice can move their nest away from the heat during recovery.

Ventilation: In our attempt to standardize lab conditions—especially with regard to our desire to keep SPF (specific pathogen free) and immunocompromised animals, as well as to minimize allergen load for animal caretakers—individually ventilated cages have been designed. These cages, although in appearance no different from the standard shoebox cages, provide a microclimate that can be carefully regulated. Cages can be ventilated with a rate up to 120 air changes per hour, reducing the need for frequent cage cleaning. However, health monitoring and inspection of the animals may be difficult, procedures and cage cleaning might be more time-consuming, and the high intra-cage ventilation rate could induce chronic stress and heat loss due to the draft (Baumans et al., 2002; Krohn, 2002).

In a personal communication on CompMed several years ago, a researcher asked advice on unexplained death of his mice who were housed in recently purchased IVC cages. Further inquiry revealed that the IVC air inlet was situated around the drinking nipple, forcing the mice to drink in a constant draft. The mice, it turned out, suffered from dehydration, either from avoiding the nipple due to the draft, or the constant drying airflow in the cage. Humans consider air speed greater than 0.2 m/s to be drafty and this is generally agreed to be an upper limit for rodents, as well (Lipman, 1999). This can be ameliorated by moving the air inlet to a different location in the cage. When the air inlet was located at the top of the cage and nesting material was provided, air changes of up to 60 per hour were tolerated, with no

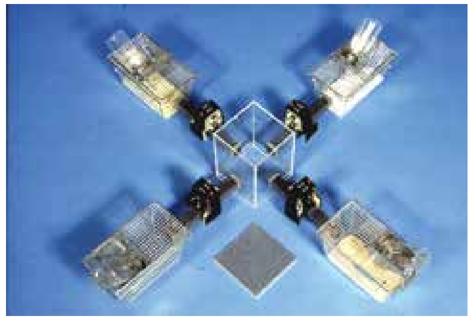
INDIVIDUALLY VENTILATED CAGE SYSTEM

adverse effects on the physiology or behavior of the mice (Baumans et al., 2002). This means that the location of the air supply to the cage (from the side or from the top), the ventilation rate, and the presence of nesting material are important considerations when assessing the impact of IVC housing on the well-being of mice. Evidence that animals are reacting to draft could be a change of location of the nest and the building of barriers of bedding.

Lights and sounds: Mouse rooms usually have a 12-hour light-dark cycle with lights off during the night and light intensity during the day as high as 300 lux. Light has damaging effects on the retina, but can be particularly damaging in nocturnal species such as mice, and is even more detrimental in albino strains, which lack pigment protection (Lanum, 1978). Therefore, it is of utmost importance that light intensity be kept to a minimum. In general, for albino mice, light intensity should not exceed 60 lux at the cage level. This can be achieved by covering the highest cage shelves and by providing mice with structures, such as nesting material, that enable them to shelter from bright light.

Mouse rooms are a constant source of sounds emitted by the mice themselves, animal technicians and caretakers, and equipment. Some of these sounds, such as equipment producing ultrasound, or sudden noises, such as doors and cages opening or closing, may be a source of stress for the animals. Chronic and/or loud noises may induce impaired behavior, cognition, and immune function in mice (Cheng et al., 2011; Pascuan et al., 2014; Tamura et al., 2012). Playing background music in animal rooms may help mask stressful sounds (Van Loo & Baumans, 2004; Alworth & Buerkle, 2013).

Structuring and size of the cages: Appropriate structuring of the cage environment is typically more beneficial than provision of a larger floor area; however, a minimum floor area is necessary to provide a structured space. This enables mice to use the vertical cage dimension as well. It is difficult to scientifically specify the minimal sizes of cages for maintaining laboratory mice, as much depends on the strain, group size and age of the animals, their familiarity with each other, and their reproductive condition (see Whittaker et al., 2012, for an excellent review). In terms of structure, the home cage can be furnished with, for example, nest boxes, tubes, partitions, and nesting material (Latham & Mason, 2004; Sherwin & Nicol, 1997; Baumans, 2005).

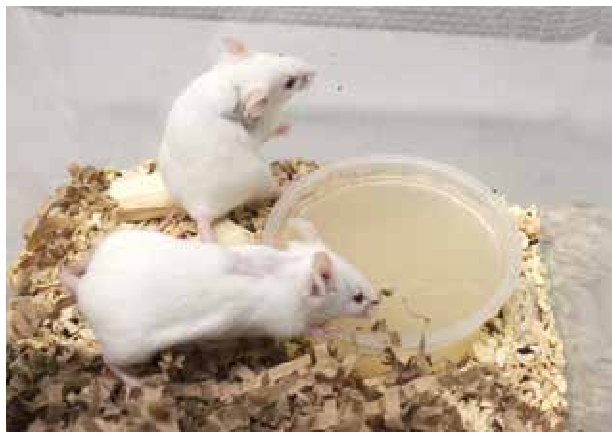


PREFERENCE TEST SYSTEM

However, provision of environmental refinement should not be a process of randomly applying objects that staff consider attractive for the animals; instead, environmental refinement should be regarded as an essential component of the overall animal care program, and equally important as nutrition and veterinary care. It is critical to evaluate environmental refinement in terms of the benefit to the animal—assessing the use of and preference for certain refinements, the effect on behavior (in particular, species-typical behavior), and the effect on physiological parameters. At the same time, it is necessary to evaluate the impact on scientific outcome, how the refinement influences the scientific study, and whether and how the statistical power is affected. Communication and teamwork among animal welfare scientists, animal research scientists, institutional animal welfare officers, veterinarians, animal ethics committees, and animal facility management and personnel is a key to success (Ottesen et al., 2004; Weed & Raber, 2005; Baumans et al., 2006). Many experiments

have been performed that reveal the mice's preferences for cage type, bedding and environmental refinement, and how this affects the animals' well-being and quality of research (see, e.g., Blom et al., 1996; Garner et al., 2004; Kirchner et al., 2012; Van Loo et al., 2005; Van de Weerd et al., 1997; Nicol et al., 2008). These studies support the now-accepted notion that controllability and predictability of the environment are highly important factors in enhancing the mice's physical and psychological well-being, by providing stimuli meeting the species-specific needs (Poole, 1997; Baumans, 2005; Van Loo et al., 2005). These needs include social contact, nest building, hiding, exploration, foraging, gnawing, and resting. Mice are highly susceptible to predation and are likely to show strong fear responses in unfamiliar situations if they cannot shelter. These responses include attempts to flee, biting when handled, or sudden immobility to avoid being detected. For this reason, cages should be provided with a shelter or hiding places. Security can be achieved via manipulable nesting material, hiding places, and compatible cage mates. Even simple environmental refinement induces a robust and replicable anxiolytic-like effect in mice (Sztainberg & Chen, 2010). Moreover, providing nesting material helps mice keep their nests clean, thus always providing them with a feces-free resting area (Godbey et al., 2011; Boivin, 2013).

Food and water provision: About 15% of the time that mice are awake is spent eating food, in numerous small bouts of feeding behavior (Van de Weerd et al., 1997). Mice feed in social bouts and learn from each other with regard to the type of food and drink that can be consumed, and how it is consumed (Baker, 1985). In the laboratory, mice may be confronted with novel ways to obtain food and water several times in their lives; for example, a drinking bottle, an automated watering system, and water-containing substances such as potatoes or commercially available gels (e.g., Solid Drink) during transport. In our experience, acclimatization within a social group to these novel ways considerably speeds up the learning curve. In studies where mice were individually housed in cages with an atypical food dispenser for metabolic measurements, they typically lost weight for up to 3 days. Through acclimatization to the food dispenser in groups prior to experiment, mice learned to eat from the novel dispenser within a day (A. M. Van den Hoek, personal communication, May 2014). Food can also be used as environmental refinement. With ad libitum food pellets readily available, foraging behavior cannot be expressed



EASILY ACCESSIBLE LIQUID-CONTAINING GELS ENSURE DEBILITATED MICE HAVE CONSTANT ACCESS TO WATER

fully. This may lead to stereotypies such as food grinding behavior. Scattering grain, as refinement, has been shown to decrease this behavior (Pritchett-Corning et al., 2013). If mice are not well—for example, when used for studying progressive disease models—providing readily available food and water via such things as lengthened drinking nipples, food pellets in the cage, and glucose-containing substances such as Solid Drink is a prerequisite not only to reducing discomfort for the animals, but also to ensuring that experimental data reflect the disease under study, rather than dehydration or famine.

Social contact: For gregarious species, such as the mouse, social contact is an important part of their environment and should only be denied in exceptional cases, e.g., extreme aggression or for scientific reasons. Provided that the group composition is harmonious, social interactions are important contributors to animal welfare. Group-housed mice are able to engage in social exploration, and the behavioral activities of one animal—such as scent marking or digging—may also be a valuable source of novelty that elicits exploration by the other individuals (Olsson & Westlund, 2007). More importantly, group-housed mice provide each other with social support (or "social buffering") when encountering a stressful situation (Hennessy et al., 2009), and several studies have suggested that mice benefit from being socially housed with respect to postoperative recovery and the need for pain relief (Van Loo et al., 2007; Pham et al., 2010; Jirkof et al., 2012).

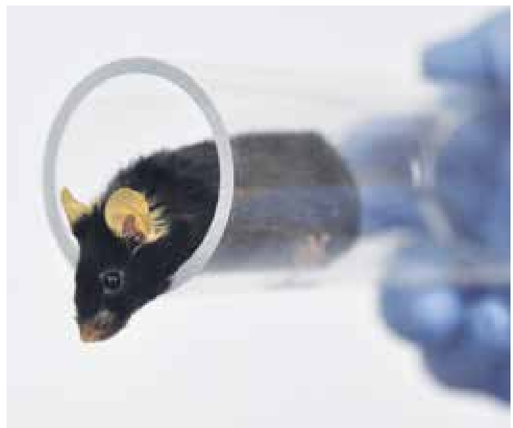
The successful establishment of harmonious single-sex groups requires the grouping of individuals who are compatible, a task that is especially challenging with male mice. Compatibility is strongly influenced by internal factors such as age, sex and hierarchical rank, and external factors such as availability and distribution of resources, and availability of space (Van Loo et al., 2003; Akre et al., 2011). The effects of space availability on the welfare of mice are not consistent. In general, aggression between male mice seems to decrease with increased crowding, however, other studies indicate that crowding increases stress-related parameters (see Olsson & Westlund, 2007, for a review). Some factors, however, can be managed by good husbandry practices, including housing mice in small, socially stable groups of three males (Van Loo et al., 2001), transferring nesting material, but not dirty bedding, during cage cleaning (Van Loo et al., 2000), and avoiding exposure of male mice to (unfamiliar) male urine (Lacey et al., 2007). Anecdotal evidence from our lab shows that housing male and female mice in different rooms, handling males before females, and generally keeping disturbances to a minimum clearly helps in reducing aggression further. This intuitively makes sense, since stressful events are known to trigger aggression (Pant & Nath, 1993). Another very interesting observation was made by P. Y. Wielinga (personal communication, September 2011). In a study in which a high fat diet was tested for its effect on metabolic parameters in male BALB/c mice, the mice fed the control diet all had to be separated due to high levels of aggression, while the mice fed the high fat diet lived peacefully together. Unfortunately, this finding has never been investigated further. It could be worthwhile investigating whether the nutritional balance for laboratory mice needs to be re-evaluated.

If social housing is not possible due to experimental restraints or excessive aggression, several other options may be worth considering. Co-housing aggressive males with an ovariectomized female as companion may be a solution when the animals have to be kept for long periods, although whether the negative impact of the surgical procedure is outweighed by the benefit of social housing needs to be taken into account. In studies with instrumented animals, non-instrumented buddies may be an option. If the instrumentation does not allow this, some authors have suggested the provision of mirrors (Sherwin, 2004; Fuss et al., 2013) or cohousing animals with a divider in between (Van Loo et al., 2007). The latter two solutions have not proven as yet to alleviate the detrimental effects of individual housing.

Experimental procedures

When evaluating the way in which different techniques are performed on mice, knowledge of their natural behavior and the way they see the world, together with a little common sense, can lead to minor adaptations in technique with decidedly positive consequences for the mice.

Training, conditioning and reward: A rather unexplored area of investigation in mice is the use of training, conditioning and reward. These are very common means of refinement for larger animals, such as dogs or primates. Training and conditioning help the animal to predict what is coming, thereby decreasing the stress response (Weiss, 1972). Adding a reward may help in associating stressful stimuli with overall positive events. Dogs and primates, for example, can be taught to readily offer their paw or arm for blood removal. For mice, the use of training, conditioning and reward is less commonly done, even though several studies show promising results with substantial effects on stress response to minor procedures, such as restraint (Meijer, 2006). The use of training, conditioning and reward for mice, therefore, is certainly worth promoting.



HANDLING OF MICE: (TOP) CUPPING IN A HAND, (BOTTOM) VOLUNTARY ENTRY INTO A TUBE

Handling and restraint: Handling is by far the most frequently performed technique on mice. Virtually all procedures involve picking up the mouse from the cage (i.e., cage cleaning, health check, and other invasive procedures). The most common method for taking mice from cages is to pick them up by their tails, usually by hand, or sometimes even with forceps. This, of course, is very unnatural for the mouse (or, to the extent it is "natural," it is like being caught by a predator). By making use of natural responses of the mouse to climb in or on things, Hurst & West (2010) have shown that picking up mice by use of tunnels or open hands led to voluntary approach, low anxiety, and acceptance of physical restraint. An important aspect here is that the mice continue to keep their feet on a base, rather than hanging from their tails, staring into an abyss. Similarly, other natural responses from mice can be used to aid restraint procedures with less stress. For example, a mouse who needs to be restrained in a tube for blood removal or otherwise could be expected to more readily do so if the tube is a dark, apparently safe haven.

Oral dosing: In studies that require daily oral dosage, voluntary ingestion via water or

VOLUNTARY CONSUMPTION OF A TEST SUBSTANCE AS AN ALTERNATIVE TO ORAL GAVAGE

a tasty gel or paste has proven to be a valuable alternative to oral gavage in mice (Zhang, 2011). Voluntary dosing is a positive rather than a stressful event. Further, the risk of mishaps related to oral gavage is absent. Mice appear to have a specific appetite for savory tastes such as peanut butter, cheese and bacon grease (Witmer et al., 2014). The use of voluntary ingestion of daily dosage is increasingly used for administration of analgesics (Van Loo et al., 1997; Pham et al., 2010; Kalliokoski et al., 2011; Abelson et al., 2012; Molina-Cimadevila et al., 2014), as well as dosing of test substances (Walker et al., 2012; Gonzales et al., 2014).

Several other procedures may be candidates for replacement with less stressful ones. For example, keeping mice in metabolic cages overnight is not necessary if single urine or feces samples are needed. Simply transferring the mouse into a plastic bucket for a short period of time will usually trigger voiding of urine and feces (Van Loo et al., 2001).

Concluding remarks

In preparing this chapter, we have tried to guide the reader into the world as perceived by mice. Being humans, we realize that in no way can we be certain that we are correct on all counts. Nevertheless, we hope that we have provided the reader with some new knowledge and a huge amount of empathy with the way laboratory mice experience the world around them. The examples we have given on ways to improve the life of laboratory mice are by no means exhaustive. Instead, we invite the reader to consider them and to continuously be aware of what we can do to make lives easier for our mice.

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Rats

Rats

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The brown rat, or Norway rat (*Rattus norvegicus*) probably originated in Asia, near the Caspian Sea, and has spread throughout the world as a human commensal (Donaldson, 1912; Hedrich, 2000). With their spread from Asia to Europe and places beyond in the Middle Ages, brown rats were quickly recognized not only as pests that had an economic impact through competition with humans for limited resources, but also as vectors of disease. Rat-catching has existed as a trade since at least medieval times; typically, these early exterminators were paid a per-rat bounty, although sometimes payment per job was arranged (Matthews, 1898). Rats were also captured for the rat-baiting trade, which rose to prominence after other popular blood sports, such as bull and bear baiting, were banned in the early 19th century. Rat-catchers may also have bred rats to increase their financial security, rather than relying solely on what nature provided, and this practice may have led to the rat fancy and rat domestication, both of which arose in Europe during Victorian times. Unusual color variants captured for rat baiting were instead saved and tamed, then sold. Although some claim that the rat was the first mammal domesticated solely for research, it is unclear if research or the rat fancy was the primary driver (Lindsey & Baker, 2006). Many books have been written on the natural history, behavior, biology, and research uses of the rat (Calhoun, 1963; Cooley & Vanderwolf, 2005; Krinke, 2000; Sharp & Villano, 2013; Suckow et al., 2006; Sullivan, 2005; Waynforth & Flecknell, 1992; Wishaw & Kolb, 2004). Brown rats are clever, social, physically robust rodents (Ben-Ami Bartal et al., 2011), who are used extensively in every aspect of teaching and biomedical research, from psychology to genetics to safety testing to infectious disease to neuroscience.

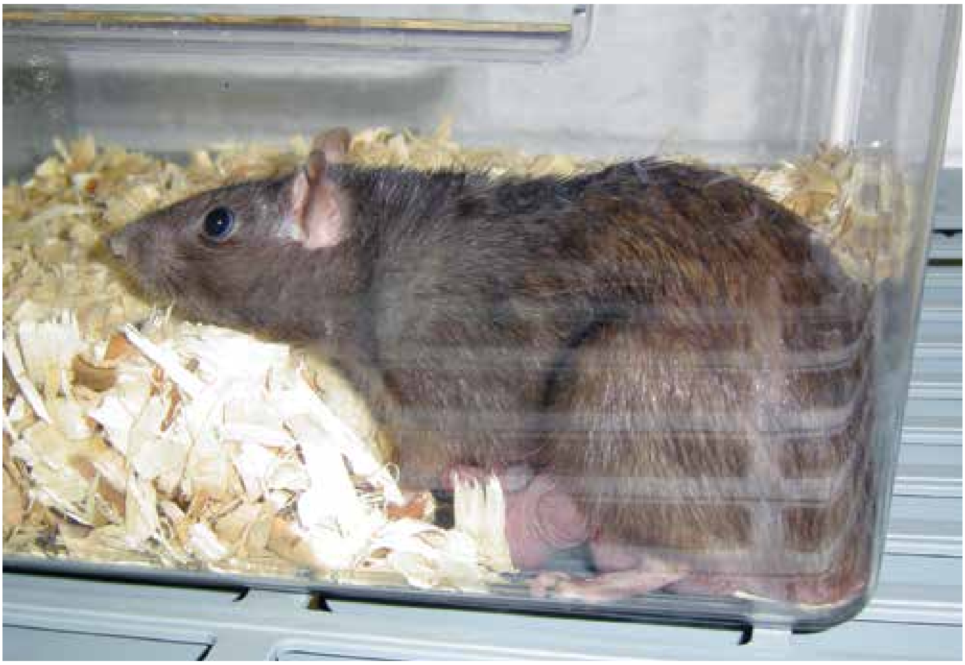
Species-typical characteristics of laboratory rats

Origin: Laboratory rats are domesticated wild brown rats with no genetic evidence of crossing with black rats (*Rattus rattus*) (Hedrich, 2006), unlike laboratory mice, which carry genes from several closely related species and subspecies (Didion & de Villena, 2013). For the rest of this work, "rat" will be used to refer, specifically, to the brown rat rather than any other rat species in use in research or found in the wild. Inbred strains and outbred stocks of laboratory rats originated in the early 20th century in the laboratories of H. H. Donaldson and W. E. Castle (Castle, 1947; Donaldson, 1912). Rats have been selected and bred by humans for various characteristics such as coat color, tumor

susceptibility, disease development, or responses to compounds (Castle, 1947). Domestic rats differ from their wild ancestors in several important ways, including greater docility, larger body size, and increased fecundity. However, if released into the wild, laboratory rats will readily revert to ancestral behaviors (Berdoy, 2003), so discussion of wild rat characteristics is appropriate when considering laboratory rats.

Behavior: Rats are social animals, living in kin groups in the wild. The kin group maintains and defends a home range that varies in size, depending on resources and availability of cover (Calhoun, 1963; Davis et al., 1948; Taylor, 1978). Above-ground movement trails are readily apparent in the environment. Residents of a home range excavate an extensive burrow system with many chambers and exits (Berdoy, 2003; Calhoun, 1963). A burrow system is typically occupied by a dominant male and related or familiar females with offspring. Low-status animals, often juveniles, are crowded into territory not claimed by a group (Calhoun, 1963). Although low-status females may breed, the matings are usually unsuccessful in producing weaned pups unless the female has a territory and burrow (Calhoun, 1963). Typically, related or familiar females will communally rear pups (Schultz & Lore, 1993). Female rats are aggressive in defense of the young and burrow. Gestation lasts 21–24 days, with larger litters generally having shorter gestation periods. Stress may lengthen the gestation period, probably through embryonic diapause (Pritchett-Corning et al., 2013).

Prior to parturition, a female typically isolates herself in a burrow and builds a nest. Rats nurse in various positions, depending on security, experience, and demands of pups. Pups play a great deal once they are mobile at approximately 12–14 days of age, and their play consists of chasing, wrestling, and pouncing (Pellis & Pellis, 1997; Vanderschuren et al., 1997; see also other work by Pellis & Pellis), all while emitting chirps that some have characterized



TWO OF THE MANY NURSING POSTURES ADOPTED BY RAT DAMS: (TOP) BROWN NORWAY RAT NURSING IN THE ACTIVE COVER POSITION, (BOTTOM) LONG EVANS RAT NURSING IN A RELAXED POSTURE

as laughter (Panksepp, 2007). Juvenile rats are weaned by the mother in a gradual process occurring between 3 and 4 weeks of age (Cramer et al., 1990) and reach sexual maturity between 4 and 7 weeks of age (Kennedy & Mitra, 1963). Reproduction may occur year round, although it declines substantially in the winter months (Andrews et al., 1972). Some laboratory rats are photoresponsive, indicating that wild rats may also have reproductive and other somatic responses to shorter days (Heideman et al., 2000; Lorincz et al., 2001; Shoemaker & Heideman, 2002).

Rats are both nocturnal and crepuscular animals. Wild rats seen above ground during daylight hours are typically under duress, either social or environmental. The mainstay of their diet is plant material, although they are omnivorous and will kill and consume insects, birds, amphibians, and other mammals (Bandler & Moyer, 1970; Kemble et al., 1985). The early evening is the peak time for feeding and foraging but there is a second peak right before sunrise. In areas of poor resource availability, they may hoard food, but in urban environments, food hoarding is apparently rare (Takahashi & Lore, 1980). Rats will transport food from exposed sources to protected areas for consumption (Calhoun, 1963; Thompson, 1948). Rat feeding patterns involve tentative approaches and repeated small samples of unfamiliar items (Barnett, 1956) until they determine that the items are safe to consume. Rats urinate and defecate near safe food sites to signal other rats (Galef & Beck, 1985; Laland & Plotkin, 1991; Laland & Plotkin, 1993). Juveniles learn foraging skills and about feeding sites from nearby adults (Galef & Clark, 1971). Adult rats modify their food choices by smelling food on the fur, whiskers, and breath of other rats, but do not learn to avoid poisoned food that way, as the preference extends to food smelled on the breath of ill rats (Galef & Wigmore, 1983; Galef et al., 1983). All rats exhibit some neophobia when exposed to unfamiliar foods (Modlinska et al., 2015), although this is readily overcome by hunger.

Norway rats are strong swimmers (Galef, 1980) but poor climbers (when compared to black rats) (Foster et al., 2011). They often assume a temporary bipedal position, stabilized by their tails, to investigate changes in their environment. Rat gaits include a walk, a trot, and a gallop, gaits common to quadrupeds, although the gallop is rarely seen in the laboratory due to the lack of predators and cage-size constraints (Gillis & Biewener, 2001). Various postures adopted by rats during intraspecies interactions are well illustrated by Grant and Mackintosh (1963) and Barnett et al. (1982).

When not investigating their environment or foraging for food, rats spend a great deal of time grooming themselves (autogrooming) and each other (allogrooming; Bolles, 1960). Autogrooming typically occurs upon awakening, as a displacement activity or when anxious, and after eating (Komorowska & Pellis, 2004). Organized bouts of autogrooming proceed from rostral to caudal, beginning with forepaw wiping of the face and finishing with cleaning the tail (Sachs, 1988). Allogrooming is often directed from mother to pup, as well as from pup to pup; in adults, it can serve to reinforce social hierarchies as well as promote affiliative behavior (Pellis & Pellis, 1997).

To delve deeper into the natural behavior of the rat, the author recommends Calhoun (1963), an extensive examination of the behavior of rats in the wild as well as in a semi-natural enclosure. To view wild rats in a semi-natural enclosure in action, the film *The Laboratory Rat: A Natural History* is recommended (Berdoy, 2003). Another overview of the ethology of the wild rat and its laboratory counterparts may be found in Würbel et al. (2009).

Senses: As with other rodents, the primary sensory modalities of the rat differ from humans, which can make it difficult for caretakers to detect environmental issues that may be disturbing rats. Burn (2008) provides an excellent review of the rat's senses. The two primary sensory modalities of rats are olfaction and touch, with hearing and vision taking a less dominant role. Olfaction is one of the primary ways in which they gain sensory input. Rats use pheromones to communicate basic information, such as gender, health, and relatedness, as well as more complex emotional states, such as anxiety (Inagaki et al., 2014). In rats, sites of concentrated pheromonal emission include the perianal region (produced by the anal sacs and feces), the preputial or urinary papillar region (produced by the preputial or clitoral glands, as well as by urine itself), the face (produced by the sebaceous glands in the whisker pads), and the pads of the feet (mediated by the plantar glands). The action of pheromones is often mediated through the vomeronasal organ, which communicates directly with the amygdala, while a typical airborne odor is recognized by the olfactory cortex. In other words, pheromones act on emotions and responses "beneath" conscious thought. Olfaction contributes to the sensation of taste as well, and rats have the same complement of taste receptors that other rodents have: sweet, sour, umami, bitter, fat, and salt (Gilbertson & Khan, 2014; Ma et al., 2007).

Rat tactile sensory input is present throughout the body, but focused on the vibrissae. Vibrissae, also known as whiskers, are specialized hair cells with a large, blood-filled sinus and representation in the somatosensory cortex. Whiskers are primarily found on the head, although some may be found on the carpus as well. Rats have two types of facial vibrissae, the macrovibrissae, the large whiskers arranged in parallel rows on the snout, as well as the microvibrissae, which are found under the nostrils and around the lips (see photo, page 21). Rats explore their environment with active whisking movements of the vibrissae (Welker, 1964), as well as head movements that help them to determine the shape, size, and texture of objects in their environment (Hartmann, 2001; Hartmann, 2011). Whiskers on the face and feet also provide information on speed and foot placement when running (Niederschuh et al., 2015; Thé et al., 2013). Whisking is consciously controlled by the animal; it is not a reflexive response to obstacles in the environment (Berg & Kleinfeld, 2003). The amount of rat cortical space devoted to input from the whiskers is roughly equivalent to human cortical space devoted to hand and finger input. In addition to the whisker inputs, rats have touch-sensitive guard hairs that detect the presence of surfaces against the body and, like other mammals, have a subset of neurons that are sensitive to stroking (Vrontou et al., 2013).

Rats are dichromats with a rod-dominated retina, as is often found with nocturnal animals. Their rods have the typical mammalian sensitivity to light, while their two types of cone cells have peak sensitivities at 359 nm (UV light) and 510 nm ("green") (Jacobs et al., 2001). Practically, this means that rats cannot perceive "red" and behave as though red objects are opaque. Although facilities do not routinely expose their animals to UV light, both LED and fluorescent light act similarly on rats' circadian systems (Syrkin, 1999). Unlike laboratory mice, where retinal degeneration genes are relatively common, there is only one strain of rats known to be blind, the RCS rat (D'Cruz et al., 2000). Rat vision would be classed as "nearsighted" by humans (distant objects are blurry), but the severity varies by strain/stock (Prusky et al., 2002). Their degree of nearsightedness would render most markers hung on walls as navigation cues for behavioral tasks useless (Prusky et al., 2002). Rat vision is also sensitive to motion, with a sensitivity 2–3 times higher than that of humans (Douglas et al., 2006).

The hearing range of rats overlaps with that of humans, although rats can hear frequencies that humans cannot. Their hearing range, as defined by sounds audible at 60 dB, is from 500 Hz to 64 kHz, with the peak sensitivity (sounds detectible at 10 dB) at approximately 4–32 kHz (Heffner & Heffner, 2007). In comparison, human peak hearing sensitivity is from 250 Hz to 8 kHz. Many of the sounds made by rats are inaudible to humans without an ultrasonic frequency converter. In addition, noise in the ultrasonic range, rarely audible to humans, can cause stress to rats and disrupt communication.

Rats apparently have one other sense that humans may not have (or may not be able to easily access)—that of magnetoreception. Based on work with mice and other rodents, it is likely that rats have the ability to detect the Earth's magnetic field (Wiltschko & Wiltschko, 2005). This may help them to navigate or to orient their nests with the Earth's axis, the importance of which is unknown.

Addressing the species-typical characteristics of rats in the research laboratory

Caging: The size of the basic home cage recommended differs slightly between the US and the EU and has changed through time.

Evolution of cage space requirements for rats through versions of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011)

Version of the Guide	*Number/weight of rats (g)*	*Housing area per animal (cm²)*	*Height (cm)*
1963	1–3/250 4–10/250	185.8–650.3/animal 185.8–464.5/animal	20.3
1972 and 1974	Up to 100 100–200 201–300 Over 300	110 148 187 258	17.8
1978 and 1980	<100 100–200 201–300 >300	110 148 187 258	17.8
1985 and 1996	<100 100–200 200–300 300–400 400–500 >500	109.68 148.40 187.11 258.08 387.12 451.64	17.8
2011	<100 100–200 201–300 301–400 401–500 >501 Mother and litter	109.6 148.35 187.05 258.0 387.0 >451.5 800	17.8

Although rats can successfully reproduce in much smaller cages than recommended (Gaskill & Pritchett-Corning, in press; Horn et al., 2012), this may be related to the fact that they are domesticated animals who have been selected for successful reproduction under various conditions and stressors. Rats show a strong preference for a larger cage, but desires for space are subservient to the desire to have conspecifics present (Patterson-Kane, 2002). Juvenile rats exhibit a great deal of active play behavior and benefit from access to more space. Basic caging should also be sized so the rat has the ability to express all natural postures. Cages are often too short for rats to fully extend vertically.

Cages with solid bottoms are preferred to those with wire floors (Manser et al., 1995; van de Weerd et al., 1996), although wire floors with resting platforms are seemingly well tolerated. When preference testing was used to determine the strength of the preference for solid-bottomed floors, results were inconclusive, as animals would work just as hard to access space to explore as to access solid-bottomed caging for resting (Manser et al., 1996). However, when animals were not asked to work for access, a clear preference for solid-bottomed caging readily emerged (van de Weerd et al., 1996). Rats exhibit signs of stress in cages with wire floors, whether large or small, if no enrichment is provided (Foulkes, 2004). One reason rats are still housed on wire-bottomed cages is to prevent coprophagy from interfering with certain types of scientific endeavor. Coprophagy may occur both through ingestion of feces found on the cage floor as well as directly from the anus (Ebino, 1993), so the utility of wire-bottomed cages in preventing all coprophagy is questionable. Transitions from solid-bottomed to wire-bottomed cages are likely to stress rats (Giral et al., 2011). Metabolism cages, with their wire floors, lack of cover, and social isolation are likely very stressful for rats (Gil et al., 1989)

Cage space recommendations for rats found in 2010/63/EU (Table 1.2 in Annex—unchanged from Table A.2 in 86/609/EEC)

	Body weight (g)	*Minimum enclosure size (cm²)*	*Floor area per animal (cm²)*	*Number of animals that can be housed in minimum enclosure*	*Minimum enclosure height (cm)*
In stock and during procedures	≤200 ≥201 to 300 ≥301 to 400 ≥401 to 600 ≥601	800 800 800 800 1,500	200 250 350 450 600	4 3 2 1 2	18
Breeding		800 Mother and litter. For each additional adult animal permanently added to the enclosure, add 400 cm²			18
Stock at breeders in 1,500 cm² cages	≤50 ≥51 to 100 ≥101 to 150 ≥151 to 200	1,500 1,500 1,500 1,500	100 125 150 175	15 12 10 8	18
Stock at breeders in 2,500 cm² cages	≤100 ≥101 to 150 ≥151 to 200	2,500 2,500 2,500	100 125 150	25 20 16	18

and data from experiments using metabolism cages should be interpreted through that filter. The observation of clinical signs in rats in toxicologic studies is not impaired by solid-bottom caging (Van Vleet et al., 2008); in fact, the only impairment in human observation of deliberately induced mild clinical signs in rats was found in wire-bottomed cages. Cage placement on a rack may also affect rat behavior and physiology (Cloutier & Newberry, 2010) and should be considered in experimental design and analysis.

Typical laboratory rat beddings include: wood shavings, wood chips, corncob processed to various diameters, cellulose, and wood pulp. Rats prefer wood-based bedding with a larger particle size (Blom et al., 1996; Ras et al., 2002). Aspen shavings were associated with a greater rate of lung pathology when compared to a cellulose-based bedding (Burn, Peters, Day, et al., 2006). Corncob bedding has been shown to affect rats' physiology with changes in estrous cyclicity associated with corn's natural estrogenic compounds, as well as disruption of slow-wave sleep (Leys et al., 2012; Markaverich et al., 2005). Being reared on corncob bedding has also been shown to reduce measures of anxiety in male rats (Sakhai et al., 2013). Cellulose-based bedding is well tolerated by rats but does not provide the absorption of some other types of bedding (Burn & Mason, 2005). Facility-wide bedding changes may be difficult to implement, since the choice of bedding is often dictated by cost or disposal concerns.

Cage cleaning affects rats by placing them in a new environment from which all pheromonal markers have been removed. Bind et al. (2013) provides a review of how lab procedures may disrupt pheromonal communication in rodents. An additional disruption is that this usually takes place during the day, when a nocturnal animal is resting (Abou-Ismail et al., 2008). If animals are kept on a reverse day-night schedule, this is of less concern. Schedules of cage cleaning are reliant on types of caging used, with frequencies varying from once every 2 weeks to three times per week for various types of solid-bottomed cages. Rat behavior is disrupted for about an hour after cage change (Burn, Peters & Mason, 2006; Duke et al., 2001; Saibaba et al., 1996), although this disruption may be related to novelty and handling rather than disruption of pheromones, as nonbreeding rats show no preference for scent-marked cages (Burn &

Mason, 2008b). Changing the cage of a rat close to parturition or with newborn pups may result in cannibalism (Burn & Mason, 2008a). Rats close to parturition and rats with new litters should be left undisturbed for as long as is feasible. It should also be noted that frequent cage changes may have an additional follow-on effect of accustoming rats to human contact and, thus, positively affecting handleability (Burn, Peters, Day, et al., 2006).

Enrichment: Before addressing recommendations, a distinction should be drawn between **E**nrichment (capital E) and standard enrichment. **E**nrichment is typically seen as part of neurobiology or psychology projects and usually involves very large cages, training or habituation to handling, multiple manipulanda, and a constant changing or refreshment of offered objects. In contrast, enrichment entails objects or interactions that should be readily provided in a standard home cage. The figure at above right shows a cage currently being used in an **E**nrichment study, while the figure at below right shows a standard enriched cage. Although advising widespread **E**nrichment would likely benefit rats in some ways, the practicalities of research make the appropriate use of rat-relevant standard enrichment more likely to benefit a greater number of rats overall (Abou-Ismail et al., 2010; Baumans et al., 2010; Patterson-Kane, 2010; Patterson-Kane, 2004). It is worth noting that spatially and socially enriched environments were once considered unreasonable for rabbits, but the shift of many institutions to larger pens and group housing has been relatively rapid. A similar shift in perspective and practice may occur with rats now that larger caging is more readily commercially available. Consideration of the relevance of the enrichment to rats is important; things humans find enriching, rats may not (Krohn et al., 2011).

Rats are social creatures and the most highly-valued enrichment is a compatible conspecific

TOP: HOUSING USED FOR ENRICHMENT STUDY (NOTE SIZE, MULTIPLE LEVELS, MANY MANIPULANDA, AND SOCIAL ASPECTS
BOTTOM: STANDARD ENRICHMENT (TWO RATS, ONE TUBE, ONE BONE, AND LONG-FIBER NESTING MATERIAL)

(Patterson-Kane et al., 2002), although a physically enriched cage for a singly housed rat may be more beneficial than a barren cage with social partners (Abou-Ismail et al., 2014). If rats are housed in stable groups, removing animals results in signs of stress in the remainder (Burman et al., 2008), illustrating that interactions with cagemates are important. It is generally agreed that rats benefit from social interactions with other rats, and that housing rats singly is stressful, although some feel that definitive data that support the stress of single housing are lacking (Krohn et al., 2006). In some cases, physiologic or behavioral differences between singly and group-housed rats are difficult to interpret (Azar et al., 2011), but in other cases, they seem to support that singly housed animals are stressed (Kruegel et al., 2014). For example, adult male rats typically maintain a breeding territory, shared only with females and their offspring, so only subdominant male rats are found in groups in the wild. Which is more stressful, being the sole dominant animal in a territory or interacting with another animal to establish a dominance hierarchy? If it is stressful to be housed with another animal(s), is it eustress or distress, and does the eustress of the dominant animal outweigh the potential distress of subordinates (Abou-Ismail, 2011b; Hurst et al., 1996)? Regardless, social animals should be allowed the opportunity to socialize and caging should be sized appropriately for housing rats in groups of two or more. Rats with implants or other modifications that might make group housing dangerous should be given extra enrichment and the ability to hide, and may also benefit somewhat from limited contact—either visual or tactile—with conspecifics (Angermeier, 1960; Hurst et al., 1997; Hurst et al., 1998; Walton et al., 1972). Some investigators are group housing rats with head implants successfully and this should be attempted when possible (Schwarz et al., 2010).

Other changes to caging are possible and are being investigated by researchers. Rats may prefer opaque caging (Cloutier et al., 2010), but the necessity of daily animal health examinations have resulted in most institutions moving entirely to clear caging. Caging that appears opaque to the rat, such as red-tinted caging, may reduce stress in rats and this is being examined by researchers; although, as with any change from "normal," changes in "normal" physiologic values may occur (Dauchy et al., 2013). Multi-level caging may be another way of increasing welfare in both singly housed and breeding rats (Wheeler et al., 2015) since rats are motivated to climb onto objects (Williams et al., 2009). For breeding pairs of rats, dams will spend time away from their pups if this is made possible by cage configuration (Cramer et al., 1990). Providing a way for lactating females to temporarily

escape pups has proven to be beneficial in other species (Buob et al., 2013; Cloutier et al., 2013; Dawson et al., 2013).

Rats have been shown to have a preference for cages with increased interior complexity (Anzaldo et al., 1994). Also highly valued by rats is a source of cover such as a shelter, hut, or box. A nest box is valued more than nesting material (Manser et al., 1998b), but if both are offered, both will be used. The type of box preferred is an opaque, thermoplastic box fully enclosed on at least four sides, with a fifth side containing a small opening (Patterson-Kane, 2003). Any shelter provided will be used, however, with the rats both climbing on top of it (if vertical space allows) and going inside. Rats prefer long paper strips for nesting (Manser et al., 1998a; Ras et al., 2002) but will also nest with paper towels or facial tissue (Bradshaw et al., 1991; Van Loo et al., 2004). Female rats are motivated to seek out nesting material and build nests as they near parturition (Kinder, 1927; Price et al., 1977), but the response of male rats to nesting material may vary by strain/stock (Jegstrup et al., 2005). Virgin rats may need to be exposed to nesting material as youngsters in order to use it effectively (Van Loo et al., 2004). Nesting material is not commonly used as standard enrichment; however it has been shown to improve rat physiology (Vitalo et al., 2009; Vitalo et al., 2012).

Some means of enriching rat cages have become standard, such as providing rats with gnawing items made of nylon, wood, or plastic (Abou-Ismail, 2011a). Although their incisors wear mainly on the occlusal surfaces, rats are motivated to gnaw and will chew objects placed in their cage for that purpose. They will also gnaw at shelters, food crocks, or other objects placed in their cages. Although rats rarely injure themselves on sharp edges they create, objects that have sharp edges from gnawing should be removed.
Other factors may need to be considered before implementing enrichments such as

foraging or running wheels. Providing rats with a foraging enrichment (food hidden under gravel in a metal dish) decreased aggression and allowed rats to perform species-specific feeding behaviors, but also increased rates of obesity (Johnson et al., 2004). Rats will spontaneously use running wheels if provided, and the frequency of use and effects on the rat and research will differ by sex, strain, and age (Novak et al., 2012). Standard housing results in sedentary rats with poorer performance on tests of agility and strength than rats housed in large pens (Spangenberg et al., 2005); running wheels may be one way to manage rats' metabolic abnormalities (Martin et al., 2010).

Refining husbandry and research procedures for rats

When considering the rat in research, it is important to acknowledge that vendors differ, transport differs, labs differ, housing differs, husbandry differs, and individual rats differ. Few of these variables can be completely controlled for, so it is important to recognize that all these aspects can affect research outcomes (Nevalainen, 2014). If thorough information is provided in supplemental materials and methods sections of published work, it may be easier to identify some of these effects so they may be examined in the future (Prager et al., 2011).

Relatively few rats are bred at institutions; most are purchased from vendors. This means that rats used for research arrive and must adjust to completely different housing types, social interactions, husbandry schedules, enrichment, and food, among other things. Recent work indicates that rats may need longer acclimation periods (up to 2 weeks) after transport than previously thought (Arts et al., 2014). Once they have arrived, animals may be identified through tail markings, tattoos, or microchips. Marking the tail with a permanent marker was found to change behavior in rats (Burn et al., 2008). Behavioral changes associated with other methods of identification have not been studied, or the results have not been published.

Sometimes immediately upon arrival, and definitely after acclimation, rats undergo research procedures such as being handled, weighed, injected, or having blood sampled. Common procedures that occur in the laboratory or animal housing room such as cage changes and weighing stress rats, but returning rats to group housing decreases the effects of this stress (Sharp et al., 2003b; Sharp et al., 2002b). Watching most research-related procedures does not disturb rats, but observing (or more likely smelling) decapitation is stressful (Sharp et al., 2002a; Sharp et al., 2003a). Rats will react negatively to certain conspecific residues such as blood or muscle, while ignoring others such as brain (Stevens et al., 1977; Stevens et al., 1973). Alarm pheromones that researchers cannot smell and alarm calls that researchers cannot hear should be considered when performing techniques in close contact with other rats. Cleaning equipment such as behavioral apparatuses used by multiple rats should include both water and alcohol-based cleaners so that scent marks and pheromones are removed.

Despite all these sources of stress, researchers and husbandry staff can also help rats acclimate to the research environment and tolerate research-related procedures. Rats can be easily accustomed to human handling, especially when young (Maurer et al., 2008). If humans interact with rats in a way similar to the way young rats interact with each other while playing (called either tickling or playful handling, as opposed to stroking), rats will also be less fearful of humans compared to rats that were not handled (Cloutier et al., 2012). Handling by humans has been investigated as a means of reducing stress associated with common research procedures. Tickling is not necessarily a better reward when compared to food or stroking after intraperitoneal injection (Cloutier et al., 2008) but if rats are accustomed to playful handling, they show less aversion to repeated intraperitoneal injections (Cloutier et al., 2014). Human interaction may be considered enrichment for singly housed rats, and group-housed rats benefit also (Cloutier et al., 2013). Rats are readily trained using operant conditioning methods (numerous online videos show pet rats performing all sorts of feats) but training of rats to perform research-related tasks, as is undertaken with monkeys and dogs, is rarely attempted. For example, rats can learn to accept oral dosing of some compounds via syringe feeding rather than oral gavage (Atcha et al., 2010). Human handling may also decrease the effects of social isolation in rats, decrease anxiety, and improve learning skills (Costa et al., 2012; Pritchard et al., 2013).

Conclusion

During the process of domestication, we have selected and bred the rats who thrived and reproduced in the limited environment provided to them in captivity. Although laboratory rats are domesticated animals, they retain behaviors exhibited by their wild ancestors; working with, rather than against, those behavioral patterns is a good starting point. Humans must also realize that the way rats perceive their environment is foreign to the way we do, and account for this difference. The square centimeters of variance in size of typically available commercial caging is probably of little importance to rats since, in all cases, it is so much less than what would be available in the wild. Rats should have solid-bottomed cages with a wood-product bedding. If animals must be kept on wire flooring, resting platforms must be provided. Caging should be large enough to allow animals to fully extend their bodies vertically. Cages should also be large enough to allow rats of any size to be socially housed, and consideration should be given to the fact that rats will willingly tolerate less space for conspecific contact. Some retreat from human view should also be available, and rats have a clear preference for opaque, enclosed nest boxes. Nesting material will also be used by many rats, as will gnawing items, and provision of those will also enhance animal welfare. Finally, gentle, considerate, consistent handling by humans will significantly decrease stress on both sides and result in both better research subjects and better research results.

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Guinea Pigs

Guinea Pigs

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Originally from South America, guinea pigs are a diurnal crepuscular species, being active in early morning and evening with intermittent periods of rest, activity, and nibbling of food during the day and night. Grass is the natural diet of guinea pigs. In the Andes, their natural habitat, they live in herds or small groups of 5 to 10 animals and exhibit a definitive social hierarchy with a dominant male and female (Berryman, 1976). They are very alert for predators and frequently seek shelter in the burrows of other animals, as well as in crevices and tunnels formed by vegetation. Guinea pigs typically live an average of 4–5 years, but may live as long as 8 years.

They are members of the rodent suborder *Hystricomorpha*, characterized by their relatively long gestation periods, the precocious state of development of their young at birth, and the membrane covering the vaginal orifice except during estrus and parturition (Weir & Rowlands, 1974). The exact time when guinea pigs were domesticated is unknown. Through domestication, guinea pigs have become less aggressive, exhibit increased social tolerance, and are less attentive to their surrounding environment than their wild counterparts (Berryman, 1976). Scent marking with urine or secretions from the perineal and supracaudal glands rubbed on the substratum reflect the animals' social status and social roles within the group. Strange individuals are identified by the absence of group characteristic scents (Reinhardt, 1971). Although they do not groom each other, they do seek out bodily contact during times of rest.

Domesticated guinea pigs are nonaggressive, docile animals, and with frequent gentle handling and petting are extremely responsive to attention. They will get to know their human caretakers and readily respond by whistling when such caretakers enter the room. Guinea pigs frequently lick human caretakers, which is often seen as a sign of affection and acceptance (Berryman, 1976).

The young are born after a relatively long gestation period of about 66 days. Unlike other rodents, guinea pigs do not build nests, as the young are born precocial. They look like small adults and begin to consume solid food the day they are born. Young guinea pigs are very active, often enjoying games of running and jumping alone or with peers, or "popcorning" as it's often called. This period of development is very brief. Females (known as sows) can successfully breed as early as 3 weeks old and give birth at the age of 3 months. The young sow is best mated at approximately 2.5 to 3 months of age. Breeding should always occur before the age of 6 months for females, as after that age, the pubic symphysis becomes more rigid, causing issues with parturition.

Males (known as boars) also engage in sexual courtship activities when they are just 3 weeks old. When the little males start courting females, they inevitably become targets of the dominant boar, who will persistently chase them. Young males will gradually become sexually and behaviorally inhibited unless they are removed from the group (Reinhardt, 1971).

The guinea pig is an extremely social species, and bonding has been shown to be very important. Both males and females placed in challenging situations show lower cortisol levels when supported by a familiar conspecific (Kaiser et al., 2003).

Females rarely engage in fighting. They have little to fight over as they neither hoard nor compete for food. Females are so tolerant of each other that they may even nurse each other's young (Reinhardt, 1971). The mothers seem to treat all newborns equally and the young will suckle off any available female, although once nursing has started, the mother will butt away any other infants that approach her. The mothers set the nursing timetable and when ready to nurse they will pace back and forth attracting the infants. Nursing ends

abruptly after approximately 10 minutes, as the nursing mother will walk away. The lactation period ends after just 3 weeks. Although females do not appear to develop a bond with their offspring, the presence of suckling young causes them to become aggressive toward strange females (Reinhardt, 1971). It is best to introduce new females to a group when there are no lactating females or suckling young present and they do not have the scent of another group on them.

Males fight viciously in the presence of females in estrus. Generally one boar takes the role as the dominant male who then monopolizes the females. This dominance will result in all other males in the group acting more like females and even cause them to stop emitting male-typical pheromones; this prevents fights between them. The dominant male will even display courtship behaviors, rather than aggression toward these males (Reinhardt, 1971). To prevent frustration and stress from being under constant inhibition from the dominant male, it is best to separate subordinate males from the group and form bachelor groups or other harems with these males.

When housed in solid-bottom pens or cages, guinea pigs do well within a temperature range of 16–24°C (61–75°F); their preferred temperature is 20°C (68°F). High temperatures of 32°C (90°F) should be avoided, as this species does not dissipate heat well and is subject to hyperthermia and heat stroke (Canadian Council on Animal Care, 1984).

Being prey animals, guinea pigs easily panic when an unfamiliar or unseen person comes into their room. Cages or pens with open sides of metal wire are recommended so that the animals have good visual contact with their environment. They will not panic when familiar caretakers enter their room, if they are able to see them. Theses enclosure

types also provide the caretakers with easy observation of the animals and better ventilation. Being heavy rodents, weighing close to 1 kg, solid-bottom caging is highly preferred to help prevent pressure sores and pododermatitis that can develop if housed on wire bottom cages (Fullerton & Gilliatt, 1967).

Adult guinea pigs measure up to approximately 30 cm in length and require at least 3 cm additional horizontal space to allow for free expression of the stretching posture. They are poor jumpers and diggers but greatly enjoy burrowing in hay. The hay provided should be soft to avoid eye injuries.

Vocalization plays an important role in the social and sexual behavior of guinea pigs. The animals have quite a repertoire of sounds; one will always hear lots of purring, squeaking, chirping, whistling, or teeth chattering in a guinea pig room. A favorite caretaker is greeted with a noisy welcome, especially if she or he brings produce or treats! Like most other rodents, though, guinea pigs are susceptible to noise stress; sudden loud noises and other stressful sounds should be minimized (Anthony & Harclerode, 1959).

"Rodents appear to prefer sheltered areas of the cage, especially if those areas have decreased light and height. Providing such a confined space within a cage might be one way to enrich the environment of rodents" (National Research Council, 1996). Guinea pigs are very easily startled. A protected, safe refuge is a basic necessity to buffer stress in guinea pigs and assure that the data collected are not compromised by stress-related factors. Guinea pigs tend to keep close to the outer cage or pen walls, as they instinctively avoid open surfaces that would expose them to potential predators (White et al., 1989).

Addressing guinea pig-specific characteristics in the research institution

When it comes to enrichment, guinea pigs present a special challenge. They do not welcome changes and react negatively toward new food types, feeders, and water containers. Enrichment may be met with skepticism or even fear. However, guinea pigs do seem to enjoy having their common furnishing moved around to different places of the cage. Positive reactions to such changes include normal play behavior and excitement when caretakers enter, comfortable appearance, and exploration of the "new" or different cage space. Conversely, if guinea pigs do become stressed by new items or the rearranging of their furnishings, they may stop eating.

Guinea pigs do well on a commercial pelleted diet supplemented daily with hay and fresh produce. Acceptable food enrichment such as hay, hay cubes, and dried corn on the cob all allow the animals to graze throughout the day, keeping them busy and offering variety. "When good quality hay is supplied the consumption of the more expensive pelleted diet is reduced and, by their vocalization when they realize that the hay is about to be replenished, the animals clearly indicate the great pleasure they obtain from eating it and burrowing in it" (Sutherland & Festing, 1987). A daily supply of hay and other preferred fresh produce is very important, as guinea pigs forage continuously and may develop habits such as chewing and eating their own hair (trichophagia) if fresh or dry grass is not available (Sutherland & Festing, 1987; Gerold et al., 1997).

When exposed to enrichment items from a young age, guinea pigs enjoy tasting everything and generally welcome things to chew on, such as treat sticks, wood blocks, or wood sticks. Guinea pigs' front teeth continue to grow throughout their lifetime, so chewing on hard items is essential. Hard pelleted diets, as well as wooden sticks or blocks, help to prevent overgrowth of their front teeth. Fresh produce is a welcome treat, and may include greens such as kale or romaine, carrots, apple, strawberries, or other such fruits and vegetables. Regular distribution of these food items help to foster a positive human-animal relationship. It is good practice to maintain a consistent

standard food selection, as guinea pigs can be rather fickle eaters who may stop eating and starve rather than accept any new food stuff.

Guinea pigs need a social environment to maintain physiological and behavioral well-being (Sachser & Lick, 1991; Fenske, 1992). Being housed with other conspecifics also helps them cope with living in confinement (Olfert et al., 1993). Compatible group- or pair-housing should be standard practice in a research laboratory. Group-housed animals should be provided a floor space of no less than 750 cm^2 for weaned, nonbreeding guinea pigs and no less than 1,200 cm^2 for breeding females. A shelter should be provided as a refuge for the animals. Such areas serve as a comfortable sleeping area or an area to give birth. Places of refuge not only provide a safe haven but also increase the usable floor space when placed in the middle of the cage where animals typically would not go. A large box with a sliding door provides an excellent way to capture an entire group for cage cleaning; the box can simply be lifted out of the cage or pen. If the box is equipped with a removable top, this can allow easy handling of a single animal for a procedure or veterinary observation (Gray, 1988).

Guinea pigs must never be kept isolated. If the research protocol requires single-housed animals, they should always have visual, auditory, and olfactory contact with their own kind (Fenske, 1992; Olfert et al., 1993). Floor space for a research protocol requiring single-housed animals should consist of an area of at least 35 x 70 cm (2,450 cm^2) so that an adult animal can stretch and turn around freely, and a refuge box can be provided. If a medical event arises that requires temporary single housing of an animal, the minimum space needed is 35 x 35 cm (1,225 cm^2) to provide normal free movement of an adult animal and locomotor play behaviors of a young animal.

With the exception of short-term experimental protocols, guinea pigs should be kept on solid-bottom caging with bedding (e.g., National Research Council, 1996). "When grid or perforated floors are used, a solid resting area must be provided" (Council of Europe, 2006) that is large enough to allow all animals to lie on it simultaneously. To maintain a hygienic cage environment, bedding should be dust-free, seasoned soft wood and changed at least twice per week. Minimum environmental and feeding enrichment should include the bedding, a hide box, and fresh, high-quality hay given daily.

Social tensions often arise from keeping several mature males together or from overcrowding; to minimize this, one mature male should be kept with three to six females and their young. At the weaning age of about 3 weeks, the young guinea pigs should be removed and kept in same-sex groups. Adolescents housed in same-sex groups do well together, provided no females are kept within visual or olfactory contact with groups of males. Exposure to the smell of female urine will turn even the most compatible males into fractious enemies who will no longer tolerate each other (Reinhardt, 1971). A mature male can be removed from a group and replaced by another male with no problem. The females will accept him with no aggression. Strange females can be introduced to a new group without causing turmoil as long as there are no nursing females present (Raje & Stewart, 2000). Thus, it is recommended that new females be introduced to a group only when young are no longer present. Individual animals can be returned to their group without overt aggression, provided they have not been scent marked by another conspecific from a different group.

Uniform lighting should be provided for all animals, and is a fundamental condition of scientifically valid research methodology (American Medical Association, 1992), assuring that no more than the minimum numbers of guinea pigs are used to obtain statistically significant research results. Multi-tier caging systems should be avoided, as the top tier casts shadows on the bottom tiers, making it impossible to assure that the lighting provides "uniformly distributed illumination" (United States Department of Agriculture, 1995; Bellhorn, 1980; Clough, 1982).

Regular distribution of food treats such as hay, fresh produce, and yogurt drops, as well as gentle handling, help guinea pigs overcome their fear of personnel. Guinea pigs should be handled "as expeditiously and carefully as possible in a manner that does not cause trauma, overheating, ... behavioral stress, ... or unnecessary discomfort" (United States Department of Agriculture, 1995). The animals should be handled gently with both hands, one firmly around the shoulder and the other supporting the hindquarters. Nervous or impatient investigators can startle and distress guinea pigs, rendering research data collected from such animals highly suspect.

Proper handling depends on the investigator rather than on the subject. "Animal care staff are expected, at all times, to have a caring and respectful attitude towards animals in their care, and to be proficient in their handling" (Council of Europe, 2000). "Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals" (Interagency Research Animal Committee, 1996). "All who care for or use animals in research, teaching, or testing must assure responsibility for their well being. ... A good management program provides the environment, housing, and care that ... minimizes variations that can affect research results" (National Research Council, 1996) and hence, reduces the number of research subjects needed to achieve statistically significant results.

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Hamsters

Hamsters

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Hamsters represent a tiny fraction of the overall number of animals used in research. Most that are used are Syrian (or "golden") hamsters (*Mesocricetus auratus*). They are descended almost entirely from a single female and 12 offspring, captured in Syria in the 1930s, with only a few additional individuals added to the lineage in 1965 and 1971 (van Hoosier & McPherson, 1987; Laber-Laird et al., 1996). The other common research hamster, the Armenian hamster (*Cricetulus migratorius*) was introduced as a research animal in 1963 as part of the USSR-USA cultural exchange program. Less commonly used species include the Chinese hamster (*Cricetulus griseus*), brought to Harvard in 1948 (Gad, 2007), the European hamster (*Cricetus cricetus*), the Siberian dwarf hamster (*Phodopus sungorus*), and the Russian dwarf hamster (*Phodopus campbelli*).

In the wild, hamsters inhabit a wide variety of climate zones and environments, from hot deserts to more temperate European zones to frigid areas of Russia. (EU Wildlife and Sustainable Farming Project, 2009). Hamsters maintain a constant environmental temperature by digging deeper burrows in the extreme climates. Burrows are almost always dug and lived in by a single animal, with no overlap between burrows. Except during estrus, female hamsters are very aggressive and will attack other animals entering their burrow. Conflict is avoided using urine and scent marking at tunnel entrances, delineating the females' receptiveness.

All hamsters share certain characteristics. Their solitary burrows contain sleeping, pantry, and bathroom areas. Typically, hamsters are active at night and can cover more than 6 miles as they search for grains, grasses, and insects (Willows Veterinary Centre and Referral Service, n.d.). Some studies of specific wild hamster populations suggest a larger diurnal component to their lifestyle (Gattermann, 2008). Regardless of time of day, behaviors outside the burrow are focused on locating food, storing it in their distensible cheek pouches, and returning the food to the burrow. In hard times more food is collected (Day et al., 1999; Garretson & Bartness, 2014). If food is plentiful, the hamster may return will only high value items in their cheek pouches to deposit in their pantry (Garretson & Bartness, 2014).

Although considered nocturnal, hamsters eat throughout the day, awakening to a state of semi-arousal to eat nearby food (Anderson & Shettleworth, 1977; Hoosier

& McPherson, 1987). This behavior can be disrupted in the research setting if a traditional food hopper (wire lid or basket) is used. This type of food storage requires the hamster to fully awaken and stand to eat, presenting a source of stress. Items on the cage floor or in a J-type hopper will allow a hamster to more easily maintain a sleeping position and not require full waking.

The carrying and manipulation of food are so ingrained, that they should be considered imperative activities in hamsters. Hamsters in the research setting, when deprived of this ability, show signs of stress and demonstrate more hoarding behavior when provided access to food (Anderson & Shettleworth, 1977).

In their natural habitat, hamsters continuously renovate the size and shape of their burrows, as they dig out new bathroom, sleeping, and eating areas. Feces are typically brought out of the burrow and deposited on the ground. Within the nesting and sleeping areas, the contents are widely varied and can include grass, their own fur and that of other animals, feathers, paper waste, bark, etc.

These species-typical behaviors bring unique challenges to maintaining hamsters in a research facility, particularly when they are housed in ventilated rack units. This technology can make providing species-appropriate housing quite difficult. The following sections will examine each component of the cage, offering suggestions to make for better housing for hamsters. While it is recognized that institutions may not be able to make all changes, many are very simple and can provide major dividends in improved enrichment and housing for the hamsters.

Cage construction and physical size

The eighth edition of the *Guide for the Care and Use of Laboratory Animals* (*Guide*) specifies that a hamster weighing more than 100 grams should be provided with a cage space of 19 inches square and a minimum height of 6 inches (National Research Council, 2011). Most ventilated mouse and rat cages are compliant with these suggestions, although ultra-high density mouse cages may be less than 6 inches in height.

The standard ventilated mouse cage, while technically compliant, is not an ideal habitat for hamsters. After deep bedding is added for burrowing, there may not be adequate head room. Further, small cages have been linked to chronic stress in the hamster (Kuhnen, 1999). If any structures are added to the cage, the floor space can become quite limited, particularly if the caging includes a wire-basket food hopper. If a ventilated mouse cage is the only option, studies have shown that hamsters prefer large quantities of nesting material, which they can shape into a nest and burrow-like structure (Hauzenberger et al., 2006). When provided with sufficient nesting material, the bedding depth appears to be less important. Examples of ample nesting material include large handfuls of shredded paper, several paper towels plus hay and straw, and a cardboard (chewable) shack plus additional nesting material. One study suggested at least 15–25 grams worth of material per hamster (Richards, 1969).

The larger, ventilated rat cages provide much more appropriate housing for hamsters. Some of these are 8 inches tall and will accommodate a larger shelter in the cage without it interfering with food and water dispensers. They also leave room for a thicker layer of bedding and possibly a J-type feeder instead of a wire-basket feeder. Since rat cages are at least twice the size of mouse cages (~150 vs 75 in^2), they provide space for clear separation of bathroom, pantry and sleeping areas, even with multiple animals in a cage.

The rat cages can also accommodate a running wheel. Since wild hamsters can range more than 6 miles per day and many studies show them to be prodigious wheel runners, a wheel can provide highly valuable enrichment for them.

Shoebox-style, non-ventilated guinea pig cages offer even more room, typically having over 200 square inches of floor space and a height of 9 or 9.5 inches. These cages offer a variety of better features for the hamster. A guinea pig food hopper is usually a J-type feeder, which allows hamsters to carry away food. The extended height offers space for a wheel. It also allows for deeper bedding and more nesting material without incurring additional risk of flooding. Structures could have two levels and the hamster could use the roof or top for additional useable space.

Other larger rodent or rabbit cages may also work well, as they will accommodate more environmental variety. Some may require modification, as hamsters caging should be solid bottomed, with no gaps that might entrap a foot. The sides should also be solid to prevent climbing and escape. These cages may be tall enough for placement of tube-style pet systems (e.g., Habitrail OVO brand), with multiple levels and designated play, eating, and rest areas.

Feeder type and location

Conflicting opinions exist regarding feeding methods for laboratory hamsters. The current wording in the *Guide* states "feeders should be designed and placed to allow easy access to food and to minimize contamination with urine and feces" (National Research Council, 2011). At the same time, there are many citations in the literature that state that provision of food on the floor is essential (Harkness et al., 2010; Phillips, 1966). For example, Charles River, Inc., the largest breeder of the Syrian hamster for research, states in their laboratory animal guide, "Hoarding is an important behavior in hamsters. If food is only provided via food hopper, then the ability to collect and hoard it in a store is lost. … Providing food inside the cage, such as on the cage floor, allows the animal to display this natural hoarding behavior" (Winnicker, 2012). Hamsters whose cache is removed will lose weight and hoard more when loose food is offered again, suggesting that maintenance of body condition is dependent on the presence of the food cache (Phillips, 1966). Another study notes that dams with litters should receive their food directly on the cage floor to prevent preoccupation with collecting food, at the expense of the litter (Harkness et al., 2010). It is also important to remember that hamsters are coprophagic and will eat feces directly from the anus throughout the day (Harkness et al., 2010).

A viable compromise can be found with a J-shaped feeder or a food bowl placed on the bottom of the cage. Both offer the hamster the opportunity to carry entire pellets to make a pantry area and hoard food. Since this behavior can result in emptied feeders, but not require additional food, the veterinarian, animal care staff, and IACUC should develop an appropriate standard to monitor the food quality and quantity in hamster cages.

If the J-feeder or food bowl is not an option at a given facility, wire-bar hoppers that are rat or hamster width (7/16 in.) are also commonly used with hamsters. These wide spaces do allow them to bite off small pieces of food, which can be carried around. When combined with placement of food on the cage floor, this arrangement can provide hamsters with adequate conditions.

Feeders that are externally mounted, so no dust or parts of pellets can fall into the cage, are not suitable for hamsters. These


TOMATOES

feeders deprive the animal of all tactile stimuli when feeding and force head-extended gnawing, maximizing stress.

Shelters, houses and tunnels (shreddable and reuseable)

An appropriate shelter or material to construct a shelter is one of the most important components of the hamster cage. Unlike mice, hamsters prefer shelters above familiar, old (dirty) bedding (Veillette & Reebs, 2010). If only a single shelter is used in a cage, it must be large enough for all hamsters to fit inside. When placing the shelter in the cage, one should ensure it does not contact the water source (to prevent flooding) and that it does not prevent free movement through the cage. If a solid shelter is provided, it should ideally have multiple levels and surfaces.

As there are many shelter types available, the first decision is whether to deploy a reusable or single-use shelter. A reusable shelter has the advantage of not needing frequent replacement, and can be tinted to allow viewing without disturbing the animals. Shelters manufactured specifically for the research environment are typically autoclavable, while shelters for the pet market are typically only dishwasher-safe. PVC tubing can be used, although it is best to consider the potential effects of PVC on a research project before using them.

Shreddable and consumable houses, shelters, or tubes have the basic advantage of not needing to be washed. The shelter is only handled once and can provide additional stress relief to hamsters simply by being chewable. Multiple single-use shelters are available from research vendors, including shacks, homes, domes, refuges, mazes, huts, and tubes made of cardboard with certificates of analysis. They vary in size, shape and number of openings, and allow the hamster to control multiple facets of the environment. The shelters are light enough to be moved around by the cage occupants. They can use it to block light, block drafts or wind, or as a step, if food or water is high up.

A key feature of single-use shelters is that the hamster is able to customize them. Some hamsters will chew additional openings into the shelter, while others may tip them over completely and build a nest on top of the shelter. Some may completely chew the shelter and create a new nest. In this respect, the single-use shelter can become an important part of any enrichment protocol.

Nesting material

Hamsters are prodigious nest builders. As such, the appropriate volume and type of material is a very important factor in the housing of hamsters. Within many research facilities, compressed pulp squares (1 in^2) are commonly used for mouse nesting material. These squares can be used for hamsters; however, at least three to four squares are necessary for each hamster. Also, the compressed nature of the squares may confuse some hamsters, so the square may need to be manually pulled apart by the caretaker.

Loose strand paper fibers offer a more dynamic nesting opportunity. This paper is more biologically relevant in its shape and characteristics. It facilitates complex nest building as it consists of long strands of loose material. Hamsters tend to carry it around in their cheek pouches and make the nest in a preferred location. They will transport it into shelters. Its crinkly characteristics make nests with walls and roofs that can easily cover the entire animal. While these materials may be preferred by the hamster, the loose nature can present a challenge when using automated bedding dispensers. Many vendors offer options to dispense these products in quantifiable amounts.

Another option is a rodent nesting sheet, which resembles tissues made of wood pulp. These sheets are 4 x 8 in., making it possible to create nests from large pieces. The sheets can also be used in most automated bedding dispensers. Other options include fleece strips, cloth squares, and paper pads. Although these are typically much sturdier than the other products, they may not be suitable for proper nest creation and burrowing. As such, they should be avoided.

In the noncertified market there are many common items that can be used. Paper towels allow for large pieces and customization. The common lab wipe makes a lightweight nesting material, again offering a large size. Both of these can be autoclaved if required. Bench paper comes in large rolls, allowing for customized strips; however, the plastic layer needs to be peeled off the back. Shredded newsprint is a viable option, but there may be unexpected effects associated with the ink.

Common sense is probably the best guide for providing nesting materials to hamsters. The nesting material should be clean, sturdy, and of sufficient volume to allow the animals to cover themselves to modify light levels, control ambient temperatures, and provide security. In group housing, the provision of enough material, presumably, will also decrease aggression by enabling more than one nest to exist.

Bedding

As hamsters are burrowing animals, the depth and type of bedding is an important consideration for their well-being. One study (Hauzenberger et al., 2006) examined stress responses in hamsters using three different bedding depths (10 cm, 40 cm, and 80 cm). The results suggested that shallower bedding levels are associated with indicators of increased

stress, such as more frequent wire-gnawing and lower body condition scores. Given that the typical bedding depth in a research facility is less than 1 cm, these findings suggest that lack of burrowing capability may be a stressor for most hamsters in research. Further, the bedding types commonly used in research (shavings, corncob, and compressed paper bits) are not conducive to creation of a burrow.

Since it usually is not practical to consider bedding depths of even 10 cm, the goal should be to provide enough bedding to enable the hamsters to "bulldoze" through the bedding (approximately 3–5 cm). Concurrent to the increased amount of bedding should be the provision of adequate nesting material, as described above.

Gnawing

The provision of hard, chewable objects provides an excellent option for hamsters to display species-typical behavior (beyond nesting and hoarding). Like most rodents, hamster teeth continually grow and must be worn down by regularly gnawing on hard items in the environment. Failure to provide opportunities for wearing down teeth can result in overgrown teeth or malocclusion, both significant health and welfare issues.

Chances to gnaw can be loosely divided into non-nutritive and nutritive opportunities. Non-nutritive options are typically favored by researchers, as these options are less likely to cause shifts in body weight, body fat, or biochemistry. Hard nylon chewing products (bones, chews, and pucks) are likely the best option for gnawing. They can be purchased with certified components, if required by the study, and come in flavored and unflavored varieties. Rat-sized products are an appropriate size for hamsters. Softer polyurethane bones and chewing toys are also available with certified components, but will not provide the same teeth-wearing

capabilities as the harder varieties. Wood chews (i.e., pre-cut blocks, manzanita sticks, etc.) are an excellent option and can also provide additional climbing opportunities. Care should be taken to ensure the wood will not interfere with the research objectives. Also, with larger branches, it is important to ensure that the sticks don't inadvertently provide a means of escape from the cage. Myriad other options are available through pet supply stores. All new toys should be carefully tested with just one or two hamsters before purchasing large quantities.

Since most non-nutritive gnawing options will last for many days (possibly over a cage change period) protocols should be developed, in conjunction with the veterinarian and IACUC, to determine when to discard the item and how to clean and sanitize it.

Virtually an endless variety of options exist to provide nutrition and satisfy the hamster's need to gnaw. Historically, these options have been avoided by researchers, due to concerns over additional calories, unaccounted trace minerals, and nonstandard components. One strategy is to use the standard diet. Scattering a few pellets on the cage floor when the cage is changed can offer hamsters a viable enrichment option. A similar strategy can be employed by putting a few pieces of dog or monkey chow in the cage. While the ingredients of these items will typically not affect the nutritional status of the hamsters, the veterinarian, IACUC, and researcher should be consulted before trying food items not specifically designed for rodents. In all cases, food pellets offer an opportunity to both hoard and gnaw food.

High-fiber food items (i.e., high-fiber rabbit food, alfalfa, timothy cubes, etc.) can offer an enrichment option, but should not be considered a gnawing alternative. The same can be said for seeds, granolas, dehydrated fruits and vegetables, and other treats. All are excellent food enrichment items and should have minimal effects on the animals or studies, when provided in moderation, but, again, should not be considered a gnawing alternative.

Running wheels

The hamster's natural foraging behaviors cause them to have a strong urge to travel. In captivity, this urge is best addressed with a running wheel.

When choosing a running wheel, it is important to use the correct size and adjust the caging to accommodate both the height and footprint of the wheel. The running wheel should be large enough for the hamster to stand on the bottom with just a small elevation in the back feet. If the wheel is too small, it will cause the hamster's back to be unnaturally flexed.

Comfortable quarters for the hamster

What does a hamster want? As discussed above, there are many ways to easily enrich a hamster's environment. Some items do appear to be more valuable than others. Hamsters prize and will work for nesting material (Jansen et al., 1969). The nesting material that produces the best nest-building potential is more prized than low-potential material. As such, paper strips, large shavings, or sheet material that offer increased size options or structure potential are the best choices. Shelters are always used when large enough. Shelters that allow the hamster to modify them (i.e., cardboard or shaped pulp) are preferable. While hamsters appear to favor very deep bedding, this is not a realistic option in most facilities. Even so, the bedding provided should at least be sufficient to enable the animals to demonstrate burrowing behaviors. Food should be provided in such a way to facilitate hoarding, carrying, and gnawing. The hamster's desire to gnaw should be addressed with items other than food, such as hard nylon chewing products. All of these options can be accomplished within any research setting.

Hamsters want to move, they are agile climbers and wheel runners. As such, a running wheel should be provided whenever possible. If there is concern about the use of the wheel, the cages can be monitored with an infrared camera at night to determine how much the wheel is used.

Point system

It should be the goal of all facilities with hamsters to provide them with the best possible environment. Determining that environment can be difficult. One approach is to use a scoring system to benchmark progress toward the ideal environment. Shown below is one example of a scoring system. For each possible area; caging, provision of food, burrowing, bedding type, nesting material, forage/food scattering, running wheel, manipulanda, and mobility options, the current situation can be ranked to get a baseline and help determine opportunities for improvement. The system allows for some fixed items, such as cage size, while still leaving room to improve overall housing by focusing on other items, such as shelter, nesting, and food.

Caging

Cage size	*Number animals/cage*	*Score*
Cages 6–7 in. tall, floor area under 80 $in.^2$	2	1
Cages 6–7 in. tall, floor area 140–243 $in.^2$	5-10	1
Cages 8–9 in. tall, floor area 140–257 $in.^2$	8-10 3-7	2 2.5
Cages 9–9.5 in. tall, floor area 200 $in.^2$	6-9 3-5	2 2.5
Cages $\geq$ 9.5 in. tall, floor area $\geq$ 576 $in.^{2\dagger}$	7-20 1-6	3 4

Provision of standard diet

Type	*Score*
Wire-bar food hopper, with no food scattered	0
Wire-bar food hopper, food scattered at cage change	2
Food hopper IVC type, low hanging, $\geq$ 7/16 in. spacing	1.5
Food hopper IVC type, low hanging, $\geq$ 7/16 in. spacing, plus scatter food on floor at cage change	2.5
J-type feeder inside cage or bowl in cage	3

Burrowing potential

Depth	*Score*
Bedding depth < 0.5 in.	0
Bedding depth 0.5–1 in.	1
Bedding depth 1–3 in.	2
Bedding depth > 3 in.	3

Bedding

Type	*Score*
Paper bedding pellet type	1
Wood pellets	1
Corn cob 1/8 in.	1
Corn cob 1/4 in.	1.5
Wood or paper chip	1.5
Mixed bedding and nesting material	2
Shavings, large	3

Shelter

Type	*Score*
No shelter	0
Reusable, plastic shelter	2
Disposable/chewable cardboard shelter	3
Wood, grass, or hay shelter	4
More than one shelter provided	1 point plus shelter-type points

Forage/food scattering

Type/frequency	*Score*
No food scattered	0
Food from standard diet scattered at cage change	1
Single nonstandard food type scattered at cage change	2
Rotating two or more nonstandard food types scattered at cage change	2.5
Scatter feeding occurs more often than just at cage changing	3

Nesting material

Type/amount	*Score*
Nesting material added is one nest square or less than 15 g	0
Nesting material is more than 15 g, but in chip form	1
Nesting material is long grained, or uses big sheets, or large shaving	3
Nesting material is long-strand hay	4
More than one type of nesting material is offered	5

Running wheel

Presence of Wheel	*Score*
Running wheel not present	0
Running wheel present	3

Manipulada/gnawing

Type	*Score*
Soft nylon bones, gummy bones for gnawing	1
Hard wood, manzanita, fruit wood gnaw blocks or sticks; hard nylon bones	2

Mobility

Device added	*Score*
No climbing or two-story options	0
Branches larger than 3 in. or tunnels flat in cage	1
Ladders, parrot toys hanging in cage	2
Branches, tunnels, other furniture, allowing two-story activity	3

A score of 9 should be considered the minimum score for an adequate environment. A score of 15 or higher would be an example of a best practice for housing hamsters.

This cage is scoring 16.5	
Caging	2
Provision of standard diet	2
Burrowing potential	0
Bedding	1.5
Shelter	4
Forage/food scattering	2
Nesting material	3
Running wheel	0
Manipulanda/gnawing	2
Mobility	0
Total Score	16.5

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Rabbits

Rabbits

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Despite the overall decline in the number of rabbits used for research over the last several decades, the number of publications citing rabbit models is steadily increasing. While rabbits are commonly used for polyclonal antibody production, they are also widely used in other research disciplines, including infectious disease, cancer, and cardiovascular disease. Domestic breeds of rabbits (including the New Zealand White (NZW), the most frequently used in research) were selectively bred from the European rabbit (*Oryctolagus cuniculus*). With the advent of the eighth edition of the *Guide for the Care and Use of Laboratory Animals* (*Guide*), many institutions are revisiting how they house and care for rabbits (National Research Council, 2011).

As institutions engage in this process it will be important to understand the rabbit's natural behavior, and design enrichment and housing to foster their optimal welfare.

Species-typical characteristics of rabbits

The catalog of normal behaviors for rabbits, both domestic and wild, includes foraging, chewing, playing, interacting with other rabbits, rearing, hiding, and resting (Myers, 1961; Vastrade, 1986; Gunn and Morton, 1995; Morton & Jennings, 2003; Held et al., 2001; Hawkins et al., 2008). Wild rabbits are most active during the dawn and dusk hours (i.e., crepuscular time frame), rest during the day, and spend most of the night foraging and grooming (Villafuerte et al., 1993; Bakker et al., 2005; Diez et al., 2005). Domesticated rabbits have retained many social behaviors from the wild (Stodardt, 1964). In North America, the most common rabbit species, cottontails (*Sylvilagus spp.*), do not build large social warrens (Crowell-Davis, 2007). In contrast, the wild European rabbit, from which NZW rabbits are descended, is a social species; these rabbits live in large warrens in groups of up to four males and up to six females (von Holst et al., 1999) with an average 89% of males and 96% of females living in groups containing at least one other rabbit of the same sex (Cowan, 1987).

Addressing the species-typical characteristic of the rabbit in the research laboratory

Rearing up: As rabbits will stand on their hind legs to survey their surroundings, cages must be tall enough to easily accommodate this behavior. This behavior can be encouraged by hanging treats or toys from the top of the cage. While providing boxes and elevated platforms to climb on increases cage complexity and addresses the need to view the environment, it does not necessarily allow the rabbit to stand fully upright.

Foraging: Food treats, specifically hay, are preferred and hold the attention of laboratory rabbits for a greater period of time than nonfood enrichment items (Lidfors, 1997; Harris et al., 2001). Providing hay is the easiest way to encourage foraging behavior. Hay, principally timothy hay, is an important component of the rabbit diet, providing the fiber necessary for normal digestive motility. Eating grasses and other greens is normally a major component of a wild rabbit's activity and the motivation to engage in this behavior in a laboratory setting is not diminished. Hay can easily serve as forage if it is spread across the cage or pen; however, it can also be suspended from hay balls or wire whisks to encourage rabbits to assume rearing postures. Providing plenty of free choice hay can also decrease barbering behavior in socially housed rabbits (Mulder et al., 1992; Bays, 2006).

Small amounts of alfalfa hay and leafy greens, such as spinach or kale, can also be great treats to encourage grazing behavior. However, one needs to be careful not to offer too many of these type of greens as they can be high in calcium, potentially predisposing the rabbit to bladder stones. Greens lower in calcium, including most lettuces, dandelion greens, leafy herbs, and stems from broccoli or celery, can be fed more frequently without this concern (Bays, 2006). Foraging for freeze-dried fruits, vegetables, or cereals is a favorite activity of rabbits in a laboratory setting (Brown, 2009). This enrichment practice can be built into daily husbandry procedures and serves the dual purpose of engaging a natural behavior and providing an early warning system for unhealthy rabbits. Rabbits who ignores their treats can be easily identified and referred for veterinary attention before the development of more severe health problems. Foods high in carbohydrates and sugar should be used sparingly as a foraging treat, as they can result in gastrointestinal distress.

Hiding: As a prey species, wild rabbits create and then live in burrows; similarly, domestic rabbits will readily utilize hiding places (Bays, 2006; Hansen & Berthelsen, 2000). These can be complex structures built into cages or simple cardboard boxes or paper bags. Being able to hide or perch also provides a mechanism for coping when scared or stressed (Buijs et al., 2011a). Perching and hiding places are particularly important for socially housed rabbits, as they provide a means to create microenvironments and escape dominant animals (Buijs et al., 2011b). One important consideration for socially housed rabbits is the provision of multiple hiding spaces so as to avoid creating a resource that can be guarded.

Chewing and gnawing: Chewing and gnawing behaviors represent up to 20% of wild rabbit activity. For domesticated rabbits, chew toys that are not easily swallowed or cannot entrap the rabbit's head or appendages can encourage this natural behavior (Lidfors, 1997; Poggiagliolmi et al., 2011). These can include untreated cardboard tubes or wood blocks; hard plastic dumbbells, kong toys, balls, or rings; or stainless steel rattles, oversized chains, or rings. Some plastic baby toys, like oversized keys, can be repurposed for rabbit enrichment. Wooden blocks or sticks, in particular, significantly increase locomotion and intake behavior (Gunn & Morton, 1995; Maertens et al., 2012).

Contrary to their often-quiet demeanor, rabbits love to make noise by flinging toys through the air. So providing toys they can pick up with their mouths and toss can be of great value

(Bradley, 2000). Toys with bells in them are also well suited for this type of noise-making.

As with any species, it is important to choose manipulanda that will not entrap the head, limbs, or teeth, and chains must be short enough that they cannot wrap around the neck or limbs of a rabbit (Shomer et al., 2001). Sharp edges and materials that can be chewed apart should also be avoided.

Social interactions: The *Guide* states that social housing should be provided for all social species unless scientific justification, veterinary concern, or incompatibility precludes it (National Research Council, 2011). The literature describing both a preference for and the benefits from social housing of rabbits is extensive (Huls et al., 1991; Brooks et al., 1993; Whary et al., 1993; Seaman, 2002; Chu et al., 2004; Nevalainen et al., 2007). Rabbits will spend a significant percentage of their resting time lying in close physical contact with another rabbit. The benefits of social housing include increased ability to cope with new stressors, increased physical fitness, decreased gastrointestinal stasis due to increased activity, and normalized physiological parameters.

Refining husbandry and research procedures for rabbits

Improving the housing environment: For rabbits up to 5.4 kg, the *Guide* specifies 4 square feet per rabbit (National Research Council, 2011). However, providing 6 square feet or more per rabbit may increase the chance of successful, stable, social housing (Wyatt, 2013). Providing hiding/escape places and visual barriers is imperative, as it allows animals to choose whether to be in visual or physical contact with conspecifics (Bauman, 2005). A hiding place should be provided for each animal in the social housing to

prevent aggression stemming from defense of the hiding place. When floor pens are not possible, commercially available double-wide cages can be used to encourage natural behaviors and facilitate the formation and maintenance of stable social pairs (Lofgren et al., 2010; Lofgren, 2014).

Rotating enrichment items every 2 weeks can help maintain novelty (Harris et al., 2001; Johnson et al., 2003). The exception to this guideline is the provision of hay, which is a preferred food treat that should be provided daily—both to engage foraging behavior and support overall digestive health. Finally, lowering maximum light levels to 60 lux and adding more natural dawn and dusk transitions to the light cycle significantly contribute to a normalized circadian rhythm of body temperature (Verwer et al., 2009).

Litter boxes have been successfully used with rabbits, reducing the need to clean the entire enclosure daily. They can easily be lifted out of the enclosure and replaced. As with food, water, and shelter, multiple litter boxes are recommended to reduce resource guarding.

Acclimating to handling procedures: Regular gentle handling of rabbits can significantly reduce the stress associated with research procedures (Swennes et al., 2011). It can also help prevent injury due to handling, such as back and leg injuries. Some studies have suggested that handling early in life can make the adult rabbits more amenable to handling (Verga et al., 2007). In a recent study, rabbits were gently handled and restrained daily for approximately 5 minutes, over a 3-week period, before being returned to their home cage and provided a food treat (Swennes et al., 2011). In the subsequent 3 weeks, rabbits who had been habituated to handling were easier to catch and restrain by unfamiliar staff persons than their unhandled counterparts. This study demonstrated that acclimation to handling reduces rabbit stress during capture and restraint. The benefits of habituation were further demonstrated in a vaccine study in

which rabbits were held in a person's lap for 5 minutes, every other day. These rabbits had an improved immunological response to the vaccine, lower physiological arousal, and were protected from the post-vaccine weight loss experienced by unhandled rabbits. In addition, the rabbits were easier to catch and engaged in more exploratory behavior (Verwer et al., 2009).

Verwer et al. (2009) also evaluated several additional changes to handling and husbandry that may be useful for reducing rabbit stress:

- compartmentalizing the enclosure when catching rabbits, i.e., ushering them into a smaller pen and then catching them rather than chasing the rabbits around the full enclosure;
- knocking before entering the rabbit room to prepare the rabbits for such entrances;
- limiting performance of husbandry and research procedures to a time of day when the animals are already active, near dawn and dusk, rather than in the middle of a rest period (Jilge, 1991);
- spacing out invasive procedures to no more than once a day, e.g., performing baseline testing the day before, rather than simply earlier on the same day as a vaccination.

Providing social housing: The presence of a conspecific is the preferred environmental enrichment for social species, as it offers ever-changing, interactive stimuli, unlike inanimate toys or static cage furniture that provide only temporary novelties (Stauffacher et al., 2001; Nevalainen et al., 2007). Additionally, companionship provides an element of control over the cage environment, which can improve an animal's ability to cope with stressors (Newberry, 1995; Garner, 2005). During preference testing, female NZW rabbits worked just as hard for limited social contact as they did for food, indicating the relative importance of social access to rabbits (Seaman, 2002). Female pair-housed NZW rabbits spend up to 88% of their time in close proximity (Huls et al., 1991; Brooks et al., 1993; Whary et al., 1993). Adult male NZW rabbits housed in side-by-side cages separated by a perforated social-access panel show a significant preference for the quarter of the cage that provides visual, olfactory, and protected tactile contact with the neighbor (Lofgren et al., 2010). Female and male NZW rabbits housed in same-sex pairs in double-wide cages beginning at weaning can remain compatible past sexual maturity (Lofgren, 2014). Occasional barbering and

aggressive behavior can be successfully interrupted with additional hay and enrichment items (Lofgren, 2014). Furthermore, males and females can be group housed in same-sex groups of 4–10 rabbits for up to several months, although males occasionally inflicted significant fight wounds upon each other after reaching sexual maturity (Wyatt, 2013).

Ideally, rabbits should be paired or grouped from weaning age. This is most easily achieved when breeding rabbits in your own facility. However, investigators often need to order adult animals from vendors. A recent pilot study with a major vendor demonstrated the feasibility and benefits of ordering pre-paired rabbits (Lofgren, 2014). Requests for pre-pairing or grouping should be made as early as possible in the ordering process. As social housing of rabbits becomes more common practice, it is likely that vendors will increasingly provide pre-paired or pre-grouped animals.

If a facility receives rabbits who have been singly housed prior to or during shipping, attempts at pairing or grouping should be made as close to weaning age as possible. Once territories have been claimed (sometimes evident through urine spraying) or patterns of aggression (such as repeated bouts of chasing) have developed, chances of successful pairing or grouping decrease (Morton et al., 1993; Lofgren, 2014). Territories have likely been staked if each rabbit has been individually housed in a given space for more than a few hours; consequently, simply combining these spaces will often lead to immediate aggression (Lofgren, 2014); therefore, introductions should only occur in neutral spaces. Unless breeding is desired, pairs or groups should be comprised of a single gender. After sexual maturity, the risk of aggressive encounters increases dramatically, making it much more difficult to safely pair- or group-house intact males (Bays, 2006). Ideally, rabbits should be spayed or neutered to both reduce the risk of common health or behavioral issues, such as uterine tumors, urine spraying, and fight wounds, and to increase the chances of maintaining a long-term, stable social group (Bays, 2006). With careful introductions and oversight, unfamiliar intact adult females may be socially housed. While intact adult males likely benefit from having some protected social interaction (for example, through a visual divider with perforations to allow nose-to-nose contact), full physical contact can result in fight behavior and significant injuries (Lofgren, et al., 2010; Wyatt, 2013). However, several academic and commercial institutions

have successfully paired or grouped adult males, particularly if paired at weaning, group composition is not changed, and abundant hide and escape opportunities are available (Wyatt, 2013; Lofgren, 2014). It is important to remember that social cohesion can break down unexpectedly, even in long-term stable groups or pairs. Thus, caretakers must be vigilant for any signs of aggression between rabbits. Decisions to separate should be made with input from research, veterinary, and animal care staff.

As stated above, it is imperative to use a neutral location for introductions when pairing or grouping; this can often be achieved with a fenced-off play area on the floor or a fresh, clean, double-wide cage (Bays, 2006; Harriman, 2008; Lofgren, 2014). Multiple hide and escape opportunities (boxes, shelves, huts, hay bales, crates or tunnels) should be available.

Aggression can increase at feeding times, so avoid introducing rabbits until after they have been fed. Additionally, provide several sources of food, particularly hay, and water (Harriman, 2008). Rabbits should be added to the pen at the same time, as staggering their entry into the pen may allow the earlier rabbit(s) to view the later rabbit(s) as intruders. Scatter foraging enrichment on the floor of the cage, so that the animals will sniff and explore the environment.

A staff member should monitor all introductions and animals should not be left alone during this process. The observing staff member should watch for interactions, including nipping, chasing and mounting. These behaviors are often part of establishing a dominant/submissive relationship and can be a necessary step for the creation of a stable pair or group (Harriman, 2008). As long as one rabbit is performing dominant behaviors and the other is running away or passively receiving the mounting behavior, intervention is not necessary. If both/multiple rabbits are engaging in aggressive behavior, usually obvious in the first 5 minutes, intervention may be necessary.

Aggression may stop when the observer enters the pen or opens the cage door. If this is the case, offer both/all animals preferred treats and gentle interaction. Soft strokes over the head and ears can mimic the nuzzling and grooming rabbits exchange when bonded, and may assist in the introduction process (Harriman, 2008).

If aggressive behavior does not stop when the observer enters the pen or opens the door, use a spray bottle to lightly spray the rabbits with water. Once sprayed, rabbits usually will discontinue the aggressive encounter and begin to groom themselves; this provides a time-out of sorts (Harriman, 2008). Eventually, just the presence of the spray bottle may remind rabbits to remain calm (Harriman, 2008). If the spray bottle is not effective at interrupting aggressive behavior, use a visual barrier, such as a clean dustpan, to separate the rabbits. Do not reach in with bare hands, as they can become the target of aggression. Protect arms and hands with thick gloves (Bays, 2006).

Daily introductions of increasing duration (often 15–30 minutes or more) for up to 2 weeks may be necessary to assure the animals can be safely left together unattended (Harriman, 2008; Wyatt, 2013; Lofgren, 2014). Rabbits who are successfully grouped will groom one another, usually over the head and ears, and will sit and lie stretched out near one another. If these behaviors are consistently observed during introductions and are stable, overnight co-housing can be attempted.

Cage dividers that allow protected physical contact, namely sniffing, are a good option for that first night together. While often a predictor of positive bonding, it is important to note that calm behavior of animals through a divider or fence does not guarantee the animals will not be aggressive toward one another once in full physical contact (Harriman, 2008; Lofgren, 2014). If animals bite each other through the divider, as evidence of trauma to the lips or nose, they likely will not pair well together (Harriman, 2008).

Social compatibility of newly formed pairs or groups can be tracked in several ways. Using colored markers on the backs of the rabbits to track interactions from a distance for the first few days will make it easier to identify individual animals and evaluate their relationships (Whary et al., 1993). Video cameras can be useful for monitoring the animals during the first few days they are socially housed (Lofgren et al., 2010). The day after the animals have had unsupervised overnight social housing, rabbits should be weighed and examined individually for bite wounds. Some fight wounds are not readily visible, so a hands-on exam is recommended. Each facility should develop their own guidelines regarding how many or what size small wounds will be tolerated while the dominance hierarchy is determined. In many cases, there will be some small nibbles. Remember to document the findings in the individual rabbit record, both for USDA compliance as well as establishing a history if an exception to social housing amendment is ultimately necessary. Once the pair or group appears stable, the typical daily health checks should uncover any alarming changes in the social rank relationships between cage companions. Social cohesion may suddenly change and it can be difficult to identify an instigating factor. Re-pairing or re-grouping may be attempted, but should follow the same steps as an initial introduction.

When group- or pair-housing is not appropriate: Single-housing of rabbits may be necessary for veterinary or officially approved scientific reasons. While individual housing does not allow for unobstructed touch, a number of cage manufactures now make cages with perforated Plexiglas dividers to allow olfactory and limited physical contact, which may be more valuable than visual contact for rabbits (Lofgren et al., 2010).

This type of housing can be paired with a playpen area to allow for animals to receive rotating opportunities for greater enrichment, movement, and social interaction. As with primates, when using dividers that allow for visual contact, keep in mind that animals need to have the ability to "escape" from their neighbor. This can be achieved by providing a visual barrier such as a shelter or keeping half the divider opaque so the animals retain the ability to choose whether to be in visual contact with their neighbor.

Being creative: In some facilities, budgeting and allocation of money for the purchase of new cages can be a multi-year process. Some lower-cost options for providing social housing include using a floor pen or an empty child's swimming pool to facilitate exercise and socialization in the rabbit room (J. Lanzim, personal communication, July 2, 2013). These areas can be enriched with foraging treats and hide/escape items, and used for exercise and socialization of compatible rabbits. Ideally, this type of pen should be used daily for 30–60 minutes, but a less time-intensive approach is to integrate this practice on cage-change day. Another budget-friendly option for improving social contact between cage neighbors is to modify existing caging with a polycarbonate vision and olfactory panel. Alternatively, cubicles, unused primate caging, cat caging, or dog kennels can be repurposed as enrichment caging or full-time housing.

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Ferrets

Ferrets

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The ferret species commonly encountered in research institutions is the domestic ferret (*Mustela putoris furo*). A related species, the black-footed ferret (*Mustela nigripes*), is native to North America and is endangered. They may share a common ancestor, the European polecat (*Mustela putorius eversmanni*) (Poole, 1972), although the exact ancestry of the domestic ferret is still unclear (Boyce et al., 2001). The ferret was domesticated at least 2,000–3,000 years ago and has become a common household pet in the last few decades because of its social and playful behavior (Boyce et al., 2001; Plant and Lloyd, 2010; Thompson, 1951). Unlike their wild polecat relatives, which are nocturnal solitary hunters (Poole, 1972; Einon, 1995), domestic ferrets demonstrate an affinity toward humans, are social, and are quite active during the day (Boyce et al., 2001; Poole, 1972).

There are numerous sources of information regarding general aspects of ferret housing and management, veterinary care, nutrition, behavior, and physiology (e.g., Boyce et al., 2001; Brown, 2003; Marini et al., 2002; Plant & Lloyd, 2010; Quesenberry & Orcutt, 2003; Fox & Bell 1998; Fox 1998). In contrast, relatively few studies or reviews describing environmental enrichment for ferrets have been published (Baumans et al., 2007; Einon, 1995). Controlled studies have primarily focused on social isolation during early life and the effects of an enriched environment on learning behaviors (Chivers & Einon, 1982; Weiss-Buerger, 1981). A larger number of studies have been performed evaluating the effects of environmental conditions such as floor space on pelt quality in mink, which have been extensively raised for fur. As mink are closely related to ferrets, some extrapolation of the findings in these studies to ferrets may be appropriate. Specific aspects of domestic ferret behavior are described in further detail below, with recommendations for supporting species-appropriate behaviors in the laboratory.

Assessment of well-being

Environmental enrichment can be defined as the provision of an environment and incorporation of practices that provide animals with sensory and motor stimulation to facilitate the expression of species-specific behavior, reduce or prevent maladaptive behavior, and promote the psychological well-being of animals (National Research Council, 2011). Physical well-being may be easier to support and measure than psychological well-being, although they go hand in hand. Altered psychological states often result in physical manifestations that

may be hard to detect. Normal behavior of an animal in a laboratory environment can best be determined by comparing it to that exhibited by (1) other individuals of the same species under the same conditions, (2) similar species also living in a laboratory, or (3) closely related species in the wild. For example, despite some behavioral differences between wild and domestic ferrets, domestic ferrets maintain instinctive behaviors, including social play, aggression, hunting, and territorial marking, as well as maternal and sexual behaviors.

In general, proper assessment of ferret well-being requires close observation of their appearance and behavior. Body posture and activity level are essential aspects of interpreting well-being in ferrets (Boyce et al., 2001). Common postures and active behaviors include sideway approaches, rolling, chasing, wrestling, and tail wagging; often, excited ferrets demonstrate behavior that resembles dance movements (Boyce et al., 2001; Schilling, 2000). In contrast, a ferret in distress is inactive, does not explore his/her environment, has an unkempt coat that appears scruffy, may have eyes that appear puffy and half closed, and if approached, may react with aggression (Wolfensohn & Lloyd, 2003). An unusual amount of time in a "slumped" posture may also be noted (Boyce et al., 2001). Other general signs of distress are similar to those expressed by other species and include restlessness, altered eating, drinking and sleeping habits and, potentially, gastrointestinal upset.

Vocalization may indicate either excitement or distress in ferrets. Sounds that indicate excitement are described as "buck-a-buck" (also referred to as "the dook" or "clucking") and a closely related vocalization called a "chuckle" (Boyce et al., 2001; Brown, 2003). A high pitch "scream" or "screech" is a clear sign of pain, anger or terror. The bark and the hiss are other sounds that may be associated with distress, fear, impatience or warning (Boyce et al., 2001; Brown, 2003).

Play and social behavior

Play is an important part of normal development and appears to contribute significantly toward the animal acquiring normal social skills. For example, play is believed to be a means for young ferrets to learn dominance behavior (Boyce et al., 2001; Gupta, 1988; Lazar & Beckhorn, 1974). Play is also related to sexual behavior; neck biting is a common play behavior in pre-pubertal ferrets, and adult male ferrets bite at the female's neck prior to mating (Fox & Bell, 1998). Young ferrets spend a significant amount of time playing with each other, and the intensity and aggressiveness of the play increases during adolescence (Baum, 1998). Play behavior continues into adulthood, although by about 4–5 years of age, playing occurs primarily in short, occasional bouts (Boyce et al., 2001). Because ferret play is quite exuberant and can be intense, it may take a few moments of observation to distinguish social play from serious aggression (Boyce et al., 2001). General play behavior, different aspects of aggressive behavior, and analysis of social play of the ferrets and related species have been described elsewhere in detail (Bunnell, 1981; Chivers & Einon, 1982; Jeppesen & Falkenberg, 1990; Mankovich, 1982; Poole, 1966; Poole, 1972; Poole, 1978). Sex differences in play have also been studied in ferrets; for example, play behavior may be more aggressive in males as compared to females. Exposure to androgens during the postnatal period influences these differences (Biben, 1982; Mankovich, 1982; Stockman et al., 1986).

As with other species, social isolation during development has negative consequences in ferrets. For example, lack of conspecific interactions was found to cause hyperactivity that persisted into adulthood (Chivers & Einon, 1982). In mink, social impoverishment has been found to induce stereotyped behavior (Bildsøe et al., 1990)—a finding that would be expected in ferrets, as well. In contrast, socially housed ferrets raised in an enriched environment were found to be superior in maze learning and reversal (Weiss-Buerger, 1981).

In addition to interactions with conspecifics, ferrets are very interactive with humans, which is one reason their popularity as companion animals has increased greatly over the past few decades (Brown, 2003). Human interaction can itself be enriching for ferrets, and regular handling in the laboratory can prevent fearful behavior and help buffer stress during research procedures (Ball, 2006).

Recommendations: Ferrets should be socially housed in an enriched environment (as described in this chapter) that encourages play behavior. This is especially important for young animals. When they are housed together, estrus females may become pseudopregnant, which does not affect their health and general well-being. Intact males should not be co-housed in the presence of estrus females due to high potential for fighting. Gentle human interaction is strongly recommended.

Burrowing, hunting, and swimming behaviors

The ancestors of the domestic ferrets probably lived in burrows, where they slept and stored prey (Lode, 1989; Thompson, 1951), and the skills and the desire to dig and store items appears to have been retained. Pet ferrets are notorious for digging into soft materials available in homes and bringing small objects to their dens (Brown, 2003; Schilling, 2000). Similarly, domestic ferrets have retained hunting instincts (Apfelbach & Wester, 1977). For example, a study by Russell (1990) demonstrated that isolated ferrets otherwise raised in enriched conditions (with a daily change of play objects) would choose the arm of a maze leading to the more prey-like play objects, were superior in capturing crickets and moving prey models, and demonstrated more elaborate prey-catching responses than those raised in an impoverished environment.

Ferrets have good binocular vision and are skilled in tracking objects that move at 25–45 cm/sec, the escape speed of a mouse (Brown, 2003). Indeed, they seem to very much enjoy the thrill of a chase. Hunting behavior can be elicited by quickly moving small objects across the floor and allowing the ferret to chase after them (Schilling, 2000).

Most authors agree that ferrets are good swimmers; however, not all ferrets seem to enjoy water to the same degree. Some publications tailored to owners of pet ferrets recommend bathing ferrets at intervals that differ according to the season, generally around every 2 weeks (Ovechka, 2002; Shefferman, 2001). Bathing too frequently may deplete the ferret pelt of essential oils and dry their skin (Schilling, 2000; Shefferman, 2001).

At the authors' institution,[1] a ferret "play cage" has been developed by repurposing a nonhuman primate cage. Minor modifications to the cage were needed in order to prevent escape (e.g., through the food hopper). The cage is supplied with a wide variety of enrichment objects such as PVC tubes, digging basins, and glove boxes, and ferrets are placed in the cage for several hours, at least once weekly. In our experience, play behavior begins immediately after the ferrets are placed in the cage and continues throughout the day, especially if the ferrets are young.

Recommendations: Enrichment devices that encourage the natural behaviors of burrowing and hunting should be provided. PVC pipes, commercially available tunnels marketed for ferrets, used (clean) glove boxes, paper bags, and cardboard tubes are all readily available materials that allow ferrets to express burrowing behaviors. Similarly, a box (such as a rodent cage) with bedding substrate or shredded paper encourages digging behavior. Hunting

[1] At the time this chapter was written, both authors were at the Yale School of Medicine.

behavior can be elicited by providing small plastic balls or other objects that can be chased, and human caretakers can also encourage hunting behavior by using mobile toys that the ferrets chase. As ferrets—like all other animals kept in research labs—tend to lose interest rather quickly in enrichment gadgets, frequent rotation of different enrichment objects is highly recommended. A swimming basin can be provided for ferrets who enjoy water, though it is recommended that ferrets be supervised while playing with water and dried when the water is removed. Keeping ferrets in play cages is the optimal housing for these very inquisitive and playful animals. If this cannot be arranged, regular rotation in a generously furnished play cage is recommended, particularly for young ferrets and adults housed long-term.

Sleeping and resting behaviors

Despite the above-mentioned activities, ferrets spend 50–75% of their day sleeping (Boyce et al., 2001; Plant & Lloyd, 2010). As ferrets age, play behavior becomes less frequent and the animals sleep more; they also tend to sleep more deeply as they get older (Boyce et al., 2001). Specific sleep patterns vary by individual and the environment; for example, sleeping habits of pet ferrets depend on the schedule of their owners (Boyce et al., 2001). Ferrets prefer to sleep in dark, enclosed areas, generally as a group (Ball, 2006; Brown, 2003; Plant & Lloyd, 2010).

Recommendations: Ferret cages should be supplied with hammocks and enclosed areas for sleeping and rest, keeping in mind that they often prefer to sleep in groups.

Maternal and reproductive behavior

Ferrets become sexually mature at about 9–12 months of age or the spring of the year after they are born (Fox & Bell, 1998; Wolfensohn & Lloyd, 2003). Females are induced ovulators and are seasonally polyestrous. Photoperiod plays an important role in ferret reproduction as estrus is triggered in females by increasing daylight. Detailed information on breeding and reproduction is available elsewhere (e.g., Bell, 2003; Fox & Bell, 1998; Marini et al., 2002).

Ferrets are altricial animals; newborn ferrets are blind and deaf at birth with eyes and ears opening on postnatal days 32–34 (Fox, 1998; Marini et al., 2002; Plant & Lloyd, 2010). Ferret mothers express typical maternal behaviors observed in other mammals (Lazar & Beckhorn, 1974). When provided with bedding material and a nest box within 8 hours of delivery, ferret mothers collect their young into the nest and provide warmth and care for them (Baum, 1998). Ferret mothers should be allowed to wean their offspring naturally. If artificial weaning is required, the kits should be at least 6 weeks old (Brown, 2003; Plant & Lloyd, 2010; Wolfensohn & Lloyd, 2003). During the pre-weaning period, interactions between the mother and kits are essential for normal social development; if kits are weaned early, they can demonstrate rough or aggressive behaviors that will likely persist unless behavioral modifications are made by human handlers (Boyce et al., 2001).

Recommendations: Periparturient females should be kept in a quiet area and provided a nest box with nesting material. The walls of the nest box should be high enough to prevent kits from climbing over it, yet low enough to allow the mother to easily enter and exit the nesting box; a height of 6 inches (15 cm) is recommended (Bell, 2003; Marini et al., 2002). Newborn ferrets should be kept with their mother for at least 6–8 weeks. If wire-bottom caging or caging not specifically designed for ferrets is used for housing, particular attention should be paid to the flooring; large grid size or large separations (i.e., larger than 1.0 x 0.5 in.) pose a risk of injury to the kits as they emerge from the nesting box (Ball, 2006; Marini et al., 2002).

Feeding behaviors and nutrition

Ferrets are obligate carnivores and thus have short gastrointestinal tracts and minimal gut flora. Because of these characteristics, they do not digest fiber well or utilize carbohydrates efficiently (Brown, 2003). Ideally, ferrets should be fed a diet specifically formulated for ferrets, although some facilities successfully maintain ferrets on feline or mink diets (Ball, 2006; Brown, 2003; Marini et al., 2002). Ferrets will eat 9–10 small meals each day if provided with free access to food (Fox, 1998). Dietary preferences are formed by 4 months of age, so introduction of new foods later in life may be difficult (Ball, 2006; Brown, 2003). Even though ferrets generally enjoy cereals and fruits, treats high in carbohydrates should be avoided or kept to a minimum. Rather, treats or nutritional supplements that are specifically formulated for ferrets or high in protein (e.g., Nutri-Cal nutritional supplement or meat-based baby food) are preferred (Ball, 2006; Brown, 2003). Such foods may be useful for distracting ferrets during medical procedures, thus decreasing stress (Quesenberry & Orcutt, 2003).

Recommendations: A diet appropriate for ferrets should be provided. Treats can be offered in moderation for enrichment and to encourage human interaction. Foods high in carbohydrates should be avoided.

Ferret-adequate housing

Ferret-specific cages are currently available and are generally very similar to rabbit cages. Either molded plastic cages or

stainless steel cages have been successfully used; galvanized metal should be avoided, as zinc toxicosis secondary to licking galvanized bars has been reported in ferrets (Straube & Walden, 1981). Both suspended and solid-floor cages are suitable, although at least one source recommends solid flooring whenever possible to allow provision of bedding material (Ball, 2006; Plant & Lloyd, 2010). Because of the active and inquisitive nature of ferrets, any housing that is not specifically designed for ferrets should be carefully evaluated for safety. Specific risks include sharp edges and potential escape routes. Ferrets are notorious for escaping; any space large enough for their heads will inevitably result in escape (Moody et al., 1985; Plant & Lloyd, 2010; Wolfensohn & Lloyd, 2003). Recommended grid size for flooring is 1.0 x 0.5 inches, or 0.25 inches if wire mesh or slatted flooring is used (Fox, 1998). A useful feature of some available housing systems is flexibility to join cages together, providing increased floor space when populations are small or when social groups are formed.

Detailed studies investigating optimal floor space for ferrets are lacking. Certainly, severe restrictions in mobility and cage space have been shown to be detrimental in ferrets and related species, resulting in skeletal changes and reduction in pelt quality in mink (e.g., Bildsøe et al., 1991; Einon, 1995). No specific guidelines or requirements for floor space are provided in the Animal Welfare Regulations or the *Guide for the Care and Use of Laboratory Animals*, but a commonly used rule of thumb is a cage size of 4 square feet (3,721 cm^2) for two adult ferrets. European guidelines describing minimum floor space for ferrets are more specific, ranging from 1,500 cm^2 for animals ≤ 600 g to 5,400 cm^2 for a female with a litter (Plant & Lloyd, 2010). Recommended temperature and humidity ranges for housing ferrets are 40–65°F and 40–65%, respectively (Fox, 1998; Marini et al., 2002).

Various enrichment items, as described above, when added to the primary or exercise enclosure, will encourage natural behaviors and provide additional resting places (beyond the necessary bedding area). A wide variety of ferret-appropriate enrichment items are commercially available from vendors of products for animals in the laboratory and pet toy suppliers, although items not specifically designed for the laboratory may need to be evaluated for their ability to withstand sanitation procedures (see below). Improvised devices such as PVC tubes for tunnels and surgical drapes for hammocks (Baumans et al., 2007) may be more affordable than commercially available devices. However, any improvised items must be carefully evaluated for safety prior to and during use, as ferrets are very inquisitive and have a tendency to chew and ingest foreign objects; foam, rubber, and other items of similar texture, in particular, must be avoided (Ball, 2006; Brown, 2003).

Sanitation of caging and enrichment objects

At the authors' institution, ferret cages are spot cleaned daily to remove fecal waste and sanitized weekly. Prior to sanitation, enrichment devices are removed from the cages and the cages are washed in a rack washer using a pre-wash, 10-minute detergent wash, acid wash, and two 10-minute rinses. Hammocks and beds are washed in a washing machine for two cycles. Enrichment devices and toys are sanitized in a tunnel washer (heavily soiled items are pre-treated prior to washing). Nonsanitizable enrichment items such as glove boxes are primarily used in the enrichment cages and are disposed of daily.

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Zebrafish

Zebrafish

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Over the past several decades, the zebrafish (*Danio rerio*) has become an important research animal model. Many of the same characteristics that make this diminutive tropical freshwater minnow a favorite of fish hobbyists have also contributed to its emergence as a model for human development and disease, toxicology, genetics, and behavior. An important factor in this emergence is the relative ease of keeping and raising zebrafish in captivity. Zebrafish are tolerant of a wide range of environmental conditions and will readily breed and produce large quantities of offspring, even under variable or suboptimal conditions. Ironically, the fact that zebrafish thrive so well in captivity has allowed researchers to overlook the importance of developing standards for care and management, based on the biology and behavior of these animals.

As the zebrafish has become more prevalent in animal research programs, there is a growing need to develop standards of care that are conducive to the well-being of these animals. Such standards will not only improve animal well-being, but will ultimately serve to improve the quality and efficacy of research in which the fish are involved.

The purpose of this chapter is to provide those charged with the care of the zebrafish in laboratory settings with recommendations for managing the fish in accordance with their species-specific requirements. These recommendations are based upon updated, biological and behavioral data in the scientific literature and the practical experience of the author.

Natural history

The ability of caregivers and managers to promote the well-being of the many captive or domesticated animals—including zebrafish—is dependent upon their understanding of the natural history of the species in question. The zebrafish is a member of the *Cyprinidae* (minnow) family of fishes, and occurs in nature across much of India, Bangladesh, and lowland Nepal (Spence et al., 2008). This geographic region is characterized by its broad diversity of habitat types and monsoonal climate with pronounced dry and rainy seasons. Zebrafish are most commonly encountered in floodplain habitats, occupying the upper to middle zone of the water column in standing or slow-moving bodies of water. They are often associated with abundant submerged aquatic vegetation, and are frequently found in rice paddies or farm ponds constructed for agriculture (Spence et al., 2007). Zebrafish are an active, shoaling species, most typically associating with each other

in small, mixed-sex schools of 5–20 individuals (Pritchard et al., 2001). The fish spawn primarily during the rainy season in shallow water, along the margins of water bodies. They are egg scatterers; the male fertilizes the eggs once the female releases them, and there is no parental care (adults will eat their own eggs if given the chance). Once fertilized, the eggs drop to the substrate where, depending on conditions, they will develop and hatch within 2–4 days. After hatching, larval fish inflate their gas bladders (an organ that controls buoyancy in fishes) by swimming up and gulping air at the surface of the water. After this, the animals begin actively seeking prey in shallow, weedy zones rich in zooplankton, their primary dietary item.

Zebrafish are typically an annual species, and reach sexual maturity within several months after hatching. Like other animals low on the food chain, their reproductive strategy is to grow up quickly and produce as many offspring as possible before they are eaten.

The above-mentioned biological factors must be taken into account when designing laboratory enclosures for zebrafish. For example, it should be possible for caregivers to exert control over water flow rates into tanks; low or no flow conditions are typically required for larval stages, whereas increased rates of flow are appropriate for adults in order to facilitate water exchange and remove solid wastes from enclosures. Enclosures should also be large enough to allow the animals to engage in normal swimming and schooling behavior. Additionally, larval and juvenile fish should not be housed with adults, to eliminate the possibility of the adults cannibalizing the younger individuals.

Species-typical behavior

Zebrafish display a rich repertoire of behaviors that are only marginally understood. Most of what is known about typical zebrafish behavior comes from observations made in the laboratory (Spence et al., 2008).

Olfaction governs many behaviors in zebrafish. They use their sense of smell to detect and discern between different dietary items (Lindsay & Vogt, 2004), distinguish kin from non-kin (Gerlach & Lysiak, 2006), and avoid predators (Speedie & Gerlai, 2008). Olfaction also plays a critical role in reproduction. It has been shown that pheromones released by the fish control mating behaviors and promote or suppress ovulation, probably depending on climatical factors (Chen & Martinich, 1975; Gerlach, 2006).

Although zebrafish are classified as a schooling species, they can be very territorial. Under certain circumstances—typically associated with competition for resources—they readily form dominance hierarchies, and aggressive interactions occur between and within sexes (Larson et al., 2005; Paull et al., 2010). Fish will compete for food or access to it (Hamilton & Dill, 2002) and spawning sites (Spence & Smith, 2005). In captive situations, these interactions can be mediated by increased fish density (within reason). In general, aggressive interactions and territorial behaviors are highest at low holding densities, and decrease as the number of animals occupying a given space is increased (Spence et al., 2008).

Water quality

Although zebrafish are exceptionally tolerant of a wide range of environmental conditions, the operational goal of those charged with their care must be the maintenance of a stable, clean, and favorable environment. Water quality and nutrition are the most important determinants of fish health and productivity. Therefore, the water in which zebrafish are housed has to be managed in such a way that it remains consistently within a specified range of chemical and physical parameters that are known to be most favorable to the species.

Recommended water quality parameters for zebrafish in the laboratory (Harper & Lawrence, 2011)

Parameter	*Target Range*
pH	Stable, within 6.8–8.5
Salinity	Stable up to 0–5 g/L
Alkalinity	Stable 50–150 mg/L
Hardness (g/L)	Stable, 75–200 mg/L
Total Ammonia Nitrogen (mg/L)	Zero
Nitrite (NO_2)	Zero
Nitrate (NO_3)	Up to 200 mg/L
Dissolved Oxygen (DO_2)	No less than 4 mg/L
Carbon Dioxide (CO_2)	No more than 20 mg/L
Temperature	Stable within 24–30°C

The first step in this process is to ensure that the source water being used in fish housing is suitable. Contaminants, such as chemicals and heavy metals, are typically removed by running source water through deionizing resins and/or reverse osmosis filters. Once impurities are removed from the water, it can be treated with synthetic sea salts and/or buffers to create water of the appropriate salinity and alkalinity.

Once the source water is prepared, the most critical challenge to maintaining the environmental quality in a fish housing system revolves around the fact that fish excrete wastes directly into the water. The primary component of this waste, ammonia-nitrogen, is toxic to the fish, and needs to be removed. Flow-through systems remove ammonia by flushing; clean water is pumped into tanks, fish excrete wastes into the water, and the effluent is flushed out. The flow is unidirectional—clean water in, effluent water out—and may be continuous or periodic. In a recirculating system, clean water is pumped into tanks, fish excrete wastes into the water, and the effluent water is pumped into a "treatment" zone where wastes are removed before the water is returned, clean, to the fish.

Regardless of which aquaculture system is employed, the goal is always the same: maintenance of the optimal living environment for the fish. While an in-depth discussion of how this is achieved is beyond the scope of this chapter, extensive reviews of the subject are available elsewhere (Harper & Lawrence, 2010; Lawrence, 2007). Many research applications and procedures require fish to be kept in static water for varying periods of time. It is critical to remember that water quality deteriorates with time, at a rate dependent on fish density—the more fish in the water, the more quickly the water quality deteriorates in static situations. Therefore, appropriate measures have to be taken to ensure that water quality is maintained for as long as fish must remain there. These include limiting densities of fish in the water, reductions in feeding, and manual water changes.

Tank materials and design

Tanks are the primary enclosure used to house zebrafish. They may be freestanding, but are more typically designed to be supported, along with many other tanks, on a rack in an application reminiscent of books on shelves. Tanks are commonly made from glass, acrylic, fiberglass, polycarbonate, or

polysulfone. Polycarbonate tanks are most commonly used, as they are both durable and withstand repeated sterilization in autoclaves. However, it is important to consider that both polycarbonate and polysulfone may leach bisphenol A (BPA), a synthetic estrogen mimic that has been shown to cause reproductive problems in various animals, including fish (Howdeshell et al., 2003; Segner et al., 2003). While it is unclear if polycarbonate tanks utilized in most commercially available zebrafish housing systems leach BPA in significant enough amounts to harm zebrafish, it is an issue that managers, caregivers, and scientists should be aware of.

Removal of solid wastes is a very important factor in tank design, as bacterial breakdown of solids (feces and uneaten food) can produce significant amounts of ammonia, which is toxic to the fish and can interfere with the previously mentioned "treatment zones" of aquaculture systems. Solid wastes are ideally removed by water flow from the tank to the filter system, although manual removal is sometimes necessary. Tank shape can facilitate waste removal. More often than not, the tanks are square or rectangular with sloped or V-shaped bottoms. Solids collect in the low part of the slope and are moved out of the tank by flowing water, where they can be flushed or siphoned out. Tanks that have "dead zones" where solids collect and remain must be avoided.

Tanks are typically transparent to facilitate unobstructed visualization of the fish by caregivers, but lids used to cover the tanks and prevent fish escape are often tinted blue or green to reduce light waves most conducive to algal growth. Algae and cyanobacteria are natural denizens of any aquaculture system, and colonize the surfaces of tanks, gutters, and piping. While these organisms are generally not harmful to the fish, they must not be allowed to grow to the extent that they prevent caregivers from being able to observe the animals.

Tank size

The size of tanks used to house zebrafish varies considerably, depending on the experimental or breeding application. Most commercial system vendors offer several different tank sizes ranging from less than 1 liter up to 10 liters. Tanks of 1 to 3 liters are normally used to house

larval stages of fish, or adult individuals, pairs, or small groups. Larger tank sizes are typically used to house multiple adults. For larval housing, tanks should be outfitted with screens to keep young fish in the tanks while allowing water and solids to flow out.

Housing densities

The welfare of zebrafish in laboratory settings is significantly affected by housing densities (i.e., the number of fish kept in a given amount of space). Recommendations for housing density are based on current understanding of the fish's behavior, particularly concerning the relationship between housing density and stress. Stress can be inferred from various data, most notably by measuring production of the stress hormone cortisol. The fish's behavior and reproductive performance are also very useful indicators of stress.

Typically, adult zebrafish show the highest levels of cortisol production in very low- or very high-density conditions. At low (< 1 fish/L) densities, adult zebrafish will spend nearly all of their time establishing and defending territories. While this behavior is natural, the intensity and frequency of these interactions are increased within the confines of a holding tank. Constant engagement in these aggressive activities is stressful for dominant and especially for subordinate individuals (Filby et al., 2010).

Aggressive interactions between fish decrease considerably as the number of individuals in a tank increase, as it becomes progressively more difficult for individuals to defend territories. Once densities reach a certain point, it is no longer "economically feasible" for fish to establish and maintain territories and so they stop doing it. Cortisol levels are lowest when fish are held under these conditions.

At high densities (> 40 fish/L), cortisol levels tend to increase as fish become crowded (Ramsay et al., 2006). This effect is more pronounced in situations where the fish are underfed or during experimental fasting, but does not appear to result in increased aggression. Generally, it should also be noted that growth rates are depressed at densities above 20 fish per liter. Thus, recommended housing densities for mature fish is within a range of 5–20 fish/liter—not too many and not too few fish per liter.

Space requirements also vary with life stage. The aggressive behaviors described above are driven by reproductive urges (competition for mates and spawning sites) and therefore only start to occur once the fish approach sexual maturity. Immature fish do not exhibit these behaviors and may be kept at densities of up to 50 individuals per liter without any negative effect on growth and survival rates.

Recommended housing densities for zebrafish in the laboratory

Tank Type	*Age of Fish*	*Density (fish/liter)*
Nursery	Up to 45 days	Up to 50
Adult community	45 days and beyond	Between 5 and 10

Sexually segregated housing

While fish kept primarily in sexually segregated groups exhibit improved reproductive performance when compared to fish maintained in mixed gender arrangements (Kurtzman et al., 2010), this strategy should be employed with caution. In favorable environments, adult female zebrafish will constantly produce eggs that may only be released during spawning. If females are not exposed to males, mature eggs are not released and must be resorbed. However, under typical laboratory conditions, the rate of ovulation in adult female zebrafish exceeds that of resorption. In some cases, this imbalance may cause the oviduct to become plugged or clogged with degenerating eggs. This condition, referred to as "egg-binding," results in chronic inflammation of the abdomen (Kent et al., 2012) and impairs the animal's well-being.

Egg-binding is more likely to occur when fish are housed in sexually segregated groups over long periods of time. Fish kept in mixed gender groups show lower rates of egg retention because females shed eggs during spawning that naturally takes place in tanks. For these reasons it is advisable to keep zebrafish in mixed gender groups for maintenance purposes, and house them in sexually segregated groups only if they are allowed to spawn on a weekly or biweekly basis.

Housing of individuals or single pairs

Many experimental conditions require that zebrafish be individually housed, or kept in pairs. Although they are a schooling species, zebrafish appear to tolerate long-term isolation, at least when measured by body condition and reproductive performance. However, without data on the effects of isolation on zebrafish stress and behavior, it is uncertain how such isolation might affect fish well-being and study outcomes. Regardless, females who are isolated should be allowed to spawn with males at least once every 2 weeks to prevent egg binding and/or reproductive senescence. The fish should not be kept in pairs for extended durations, as a dominant-subordinate relationship may be established, leading to constant and intense aggression. Subordinate partners are at risk of being subjected to chronic stress, manifesting in reduced growth, impaired reproductive function, and increased susceptibility to disease, or they may even be killed by the dominant partner. Thus, zebrafish should only be kept in pairs when absolutely necessary. In experimental circumstances that do require pair-housing, the animals should be kept in this situation for no more than 7 days, and shelter (see below) should be placed in tanks to provide subordinate animals with refuge.

Well-being and environmental enrichment

There are three basic approaches that can be employed to assess the well-being of zebrafish: behavioral cues, performance, and physiology. Careful observation and understanding of fish behavior is a simple and straightforward way to assess welfare. Like all animals, zebrafish will display species-typical behaviors that are indicative of their well-being. Certain behaviors can be considered "normal"—that is, animals displaying them are unlikely to be distressed or experiencing adverse conditions; other "maladaptive" behaviors show that the fish are not able to adapt to a given situation, hence are distressed.

A list of normal and maladaptive zebrafish behaviors is shown on the opposite page. Caregivers should be trained to recognize and distinguish between these behaviors so that they can quickly react to adverse conditions that jeopardize the animals' well-being.

Typical behaviors of zebrafish in laboratory settings

Behavior type	*Normal behavior*	*Abnormal or maladaptive behavior*
Swimming	Moderately paced, constant	Darting sharply, erratic, freezing
Position	Generally parallel to surface	Head up, tail down (perpendicular to surface)
Distribution in water column	Throughout	Concentrated along bottom or top
Schooling	Loose	Tight
Aggression	Occasional chasing, displaying, biting	Constant chasing, displaying, biting
Feeding	Active, consuming all available feed presented at each feeding	Limited or no response to feed when presented
Spawning	High activity clusters of males and females in corners of tanks near surface, particularly early in the morning	No apparent clustering or grouping of males and females in tanks, increased aggression during morning hours
Ventilation rates	No discernible or only occasional movement of operculum (gill covering)	Rapid, constant movement of operculum

Performance indices (i.e., growth, survival and reproductive rate) are commonly used as surrogate measures of well-being in zebrafish (e.g., Castranova et al., 2011). In general, one can infer that the welfare of fish is good under conditions that also support normal growth and survival rates, and normal reproduction. Conversely, it is reasonable to conclude that welfare may be poor when performance is depressed in one or more of these areas.

A more objective way of assessing welfare in zebrafish is to monitor physiological indicators of stress, reproduction, and health in individuals or groups of animals. Elevated production of stress hormones and decreases in sex steroid production and immune response are negative indicators of welfare, while normal levels of stress hormones and basal or increased levels of steroid production and immune response metrics are considered neutral or positive. The context of each particular analysis has to be taken into account when interpreting these kinds of results.

The most comprehensive way to measure the well-being of zebrafish is to combine behavioral observations, performance, and physiological assessment into a single analysis. Perhaps the best-known example of this in the literature is a study by Filby et al. (2010) that characterized the consequences of social status in zebrafish. The social status of individual animals in groups of zebrafish was defined first by behavioral observations (dominant vs. subordinate), and then correlated with performance (growth, reproductive output) and physiological indicators (cortisol, sex steroid production and immune function).

While there are no established standards for providing environmental enrichment for laboratory zebrafish, there are a number of simple options described below. Even though they have not yet been tested, it is reasonable to assume that they do foster the well-being of zebrafish in the research laboratory.

Diet: While captive or domesticated zebrafish will accept a wide variety of different feed types, in the wild, they are primarily zooplanktivores (Spence et al., 2007). This becomes evident in the laboratory, as the fish show superior growth and survival when their diets are comprised of live zooplankton such as *Artemia*, rotifers, or *Paramecium* (Harper & Lawrence, 2010). Furthermore, because the provision of these organisms in the diet allows for the fish to engage in species-typical foraging behavior, their inclusion can be considered a form of environmental enrichment.

It should be noted that live feeds present a potential biosecurity risk, and may be considered a source of nonprotocol induced variation in specific research protocols.

Lighting/Photoperiod: Zebrafish are considered diurnal, and are primarily active during the daylight hours. Some behavior, particularly spawning, is most intense at dawn and to a lesser extent in the evening (Harper & Lawrence, 2010). Zebrafish do sleep primarily at night. This circadian pattern of activity determines many biochemical, physiological, and behavioral processes in the animal, and therefore must be maintained for captive fish.

The fish should be housed in rooms with a controlled photoperiod (usually 12–14 hours light: 10–12 hours dark). While the provision of regular light/dark periods in and of itself cannot be considered a form of environmental enrichment, the manner in which it is administered can have an effect on fish well-being. Special light controls have been implemented at the author's facility to slowly ramp lights up to full intensity (54–354 lux) in the morning and slowly ramp them down to dark in the evening. This simulation of dawn and dusk can be considered a form of enrichment and should be employed when possible.

Plastic plants: In nature, zebrafish are associated with abundant aquatic vegetation (Engeszer et al., 2007; Spence et al., 2006). The fish like to utilize plants as cover and protection from predation, and during spawning and oviposition (Spence et al., 2007). In the laboratory, zebrafish display a preference for structured environments

(containing vegetation) when given a choice (Kistler et al., 2011). While the provision of live plants in zebrafish holding tanks would probably not be practical due to perceived maintenance and biosecurity issues, plastic plants should be available for fish as an essential form of environmental enrichment. This enrichment approach serves several purposes. First, the inclusion of artificial plants in housing tanks provides subordinate fish with a refuge from aggressive or dominant fish. This is particularly important when fish are kept in pairs, or at low densities. The provision of plastic plants in housing enclosures also stimulates natural spawning and enhances cycling of eggs by females when males and females are housed together in groups. As discussed earlier, this is a simple maintenance strategy that helps prevent egg retention and binding in females that may sometimes lead to health problems. Plants may also be added to breeding tanks to enhance egg production.

There are many types of plastic plants that can be readily purchased from aquarium suppliers. The best designs are those that float and extend well beneath the surface of the water so that the fish can swim through and easily maintain position within them. Preference should be given to plastic plants that are easy to remove and can be sanitized. Further, plants with loose parts should be avoided to prevent clogging of filters.

Handling

Many experimental applications in which zebrafish are used imply frequent handling and manipulation of the animals. While most domesticated strains of fish used in the laboratory tolerate these disturbances, investigators and caregivers must be aware that these activities are always stressful for the animals. Therefore, handling should be minimized as much as possible and it should always be performed efficiently and expediently.

The skin of all fish is coated with a protective layer of mucus (i.e., “slime coat” or “slime layer”) that acts as a barrier against infection and helps the animal to maintain blood salt balance. Therefore, protective measures must be taken during handling to prevent damage to the mucus, including the use of soft nylon nets, and keeping the skin of the fish moist when they are removed from the water. Oils, soaps, and lotions damage the slime coat, and so people should wash and rinse their hands thoroughly prior to handling the animals. Zebrafish also possess specialized cells in the skin that release an alarm pheromone into the water when the protective layer of mucus is damaged during injury. When other fish sense this pheromone, it elicits a strong escape response that includes rapid darting, usually at or along the bottom of the water column. Caregivers should be aware of this, as its occurrence after procedures is a sign that the animals were improperly handled.

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Frogs

Frogs

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Although there has been a recent surge in biomedical research with frogs, they have a long history as research subjects, across many scientific disciplines. The most common frogs in research are those of the genera *Bufo*, *Hyla*, *Rana* (*Rana pipiens*, *Rana catesbeiana*, etc.), and *Xenopus* (*Xenopus laevis* and *Xenopus tropicalis*). Clawed frogs (*Xenopus* spp.) were used in a method of early pregnancy detection (Bellerby, 1934). Northern leopard frogs (*Rana pipiens*) have been involved in neurology studies since the 1950s (Fatt, 1952), and American bullfrogs (*Rana catesbeiana*) have been subjects of physiology research and teaching. Although frogs are less frequently found in classrooms today due to availability of computer models, many people can still recall frog dissection as a standard teaching tool within basic biology curricula. Northern leopard frogs (*R. pipiens*) enabled Briggs et al. (1952) to conduct their early work of cloning via somatic cell nuclear transfer, which was later used in the better-known cloning of Dolly the sheep (Campbell et al., 1996).

More recently, frogs have been widely used in genetic and developmental research. This is in large part due to their fecundity. For example, due to the large number of eggs produced over a lifetime (about 5,000) and the large egg size (about 4,000 times the size of a mouse egg), each *Xenopus laevis* female can produce approximately the same amount of embryologic material as 10^6 mice (Gurdon, 2002). Klein et al. (2002) described a National Institutes of Health initiative to create catalogs of genetic and genomic information for both *X. tropicalis* and *X. laevis* to aide in further research using these models. Other research with frogs includes the use of bullfrogs in ecological studies (Halverson et al., 2006; Laurila et al., 2006), and examination of various compounds produced in skin secretions for their antiviral, antifungal, or antibacterial properties (Mangoni, 2006). Until recently, most laboratory frogs were obtained from the wild. While these frogs were less expensive than captive-raised, there were many issues of local population impacts and unknown health status. Therefore, if frogs are to be used in research or in teaching, care should be taken to acquire only healthy, captive-bred animals from reputable breeders and suppliers.

Species-typical characteristics of frogs

Frogs, of which 6,800 species have been identified, are ectothermic (cold-blooded) tetrapod amphibians with large eyes on short heads attached to compact bodies by little or no neck. Their specialized hind legs end

in five toes that may or may not be webbed, and are adapted for hopping, jumping, running, climbing, swimming, or burrowing (Dodd, 2013). Since a discussion of all frog species used in biomedical research is beyond the scope of this chapter, the focus will be on the most common laboratory frogs.

Among the more commonly used laboratory species, the American bullfrog is semi-aquatic, with legs built for jumping great distances. Adult size is reported to vary greatly in different geographies, with a minimum snout-ischium length of about 95 mm in males and 108 mm in females (Howard, 1981). Clawed frogs are completely aquatic, with webbed toes containing claws that are well adapted for swimming. Tropical clawed frogs (*X. tropicalis*) are smaller than the African clawed species (*X. laevis*), with a typical male/female length of 36/50 mm for the former, compared to 82/110 mm for the latter.

Bullfrogs are typically found in warmer, stagnant grassland ponds, marshes, lakes, and streams with dense vegetation. This is somewhat different from ponds used by African clawed frogs, which are usually devoid of any higher plant vegetation, and covered in green algae. As all frogs are ectotherms, their activity is more reliant on ambient environmental temperature and humidity than the time of day.

Breeding and feeding activities tend to take place at night, when the air temperature is cooler and the humidity is higher. During the warmer daytime temperatures, activity tends to revolve around maintaining body temperature, either by basking in the sun or cooling under rocks or in the shade. Further, the activity of an individual species can vary widely, based on the available microclimate. For all extra-tropical species, a common seasonal theme is to maintain activity throughout warmer months and to then enter into torpor

during the colder months. This is achieved by self-burial into mud, pond caves, or shallow hiding places.

A common characteristic shared by most frogs is the existence of a fully aquatic larval stage, often referred to as a tadpole. Tadpoles will typically lack legs, have tails for swimming, gills for respiration, and smooth, moist skin that allows water to freely enter and waste to be expelled.

This larval stage varies greatly in length of time, both within and between species. The metamorphosis to adult in the American bullfrog can be as early as a few months to upwards of 3 years. *X. laevis* have been shown to develop in the laboratory setting at 10–12 weeks (Bles, 1905).

As is typical across most of the species, all North American frogs possess lungs, but also exchange gasses across their thin skin membrane. This skin is protected from trauma and pathogens by a layer of slimy mucus, though since their skin is only semi-impermeable to water loss, they must remain in moist conditions to prevent desiccation.

In some species, toxic or noxious secretions for defense against predators will also be secreted by granular glands and exist in the mucus layer on the skin surface.

Of the species commonly found in laboratories, tadpoles are herbivorous and tend to eat algae and aquatic vegetation, while adults are carnivorous and will readily eat most invertebrates and smaller vertebrates. American bullfrogs will attack any animal smaller than themselves, and will readily eat mice, snakes, or other frogs. "In *Xenopus*, prey capture employs a combination of toothed jaws that improve the grip on the prey, forelimbs that are used to fork the prey into the mouth, and the strong hindlimbs that can be used to rake the prey with the sharp claws. This shredding action enables *Xenopus* to tackle larger food items than could otherwise be ingested whole; indeed, groups of *Xenopus* may attack the same prey and can tear the body into fragments that can then be ingested. This method of feeding is particularly useful for scavenging" (Tinsley et al, 1996).

Frogs are polygamous, with males calling to attract females to a favored mating site. American bullfrogs become extremely aggressive during the mating season, attacking all other males until a female follows to the

egg-laying site. Ryan (1980) describes this as a resource defense polygyny, indirectly gaining access to females through defense of a critical resource, while Emlen (1976) describes it as a lek, or defending communal display grounds. Male clawed frogs produce mating calls through rapid contractions of intrinsic laryngeal muscles, as they lack the vocal sac found in most frog species. When the female hears these calls, she will respond with a "rapping" acceptance call or a "ticking" rejection. Regardless of attraction technique, acceptance typically leads to the male grasping the female in amplexus, the female releasing the eggs, and external fertilization occurring in the water.

Addressing the species-typical characteristic of frogs in the research laboratory

As virtually all frog species used in research are aquatic or semi-aquatic, one of the most important considerations in their laboratory care is the quality of their water. It is important to provide water that is clean and of the correct temperature, pH, alkalinity, oxygen level, and other parameters. "Standards for acceptable water quality, appropriate parameters to test, and testing frequency should be identified at the institutional level" (National Research Council, 2011, p 78), by those knowledgeable of the natural habitat and history of the specific species. Green (2010, p 36) provides a table of values for *Xenopus laevis*; she recommends 17–24°C, 6.5–8.5 pH, 500–3000 μS conductivity, 50–200 mg/L ($CaCO_3$) alkalinity, and >7 mg/L dissolved oxygen; she also stresses the need for all chlorine or chloramines to be removed. These parameters can also be followed in a typical laboratory if setting general levels across multiple species, though further investigation may be warranted for housing more exotic frogs. Currently, requirements for space and tank densities for frog species are not well established, but should account for the behavioral needs of the species. Semi-aquatic species, such as bullfrogs, must be provided with both a terrestrial space to bask and feed outside of the water, as well as sufficient water to allow them to submerge and hydrate. Tank height must also account for jumping, and not allow for escape, or injury on lids. *Xenopus* spp. are completely aquatic and have typically been housed at 2 liters of water per frog (National Research Council, 2011, p 83), though different systems allow variable housing densities. In their natural habitat, the common laboratory species interact exclusively for mating; however, most frogs are typically tolerant of each other and can be housed in groups, given sufficiently sized tanks—the exception being male bullfrogs during the mating season.

Frogs will spend a vast majority of their time remaining still and under cover to keep alert for potential predators, as well as potential prey. As such, their tanks should be provisioned with ways to remain hidden.

Opaque or translucent tanks are recommended, as well as PVC tubes or other shelters. Faux foliage can be used to float on top of the water to provide a barrier at that level, which is also easily moved for observation. If faux foliage is used, it should be cleaned when the tank is sanitized, in a disinfecting level (per label) bleach solution, followed by a very thorough rinse in clean, unchlorinated water.

Most of the frogs species used in research will readily consume a pelleted diet designed for them. Crickets and mealworms are also easily obtained and maintained sources of live feed, allowing for typical hunting behavior. For many aquatic species, it is critical to schedule cleaning soon after feeding, as residual food very quickly produces molds and an uninhabitable environment.

Handling of frogs should be carefully considered, based on the species, and kept to the minimum required. Gloves are suggested, as a means of minimizing the opportunity for contact with toxins and poisons in the protective mucus layer and to prevent damage to the layer. Also, gloves prevent the spread of *Salmonella* infections, as the *Salmonella* bacteria are frequently harbored by frogs. Importantly, Gutleb et al. (2001) suggests that latex may be toxic to some species, and any gloves used must certainly be of the powder-free variety. The careful and proper use of a suitably sized net can also reduce handling concerns. Nets should be deep enough to encase the entire frog, and have an opening sufficient to allow entry without causing trauma. A slow scooping motion should be used, taking care not to startle any frogs, as they may injure themselves or others while trying to escape.

Refining husbandry and research procedures for frogs

As little is known regarding appropriate tank densities and the effect of group housing on an otherwise solitary species, more research in this area must be done. This is also true regarding what may be the minimum space requirements that would allow adequate space to swim for all species and adequate space to jump for some semi-terrestrial species. A reliable method for identifying individual frogs also requires further investigation. Due to their delicate skin membrane, most marking systems commonly used in other species, such as tattoos or dyes, are largely ineffective, temporary, or unsafe. While toe clipping is becoming less acceptable in rodent species, it is, unfortunately, still fairly common in frogs. Other methods, such as transponders, are being explored, though a primary "best practice" recommendation has yet to be identified.

Furthermore, it is not a common practice in the laboratory to allow frogs an opportunity to experience the biologically typical state of torpor, or hibernation, by means of lowering temperatures to the point that this is induced. This may be standard practice in some systems, but it is far from universal. The effect of allowing or preventing this is not known, and warrants further study.

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Cattle

Cattle

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Cattle in the research environment can be easily cared for and managed with attention to a main principle of cattle behavior: herd-based living. Providing cattle with access to their herd-mates should be the main focus in providing them with a positive, cattle-appropriate environment not only on the farm but also in the research setting. Through an understanding of the animals' natural social system and its details, species-adequate management of cattle can be achieved. In addition to an understanding of the social behavior of cattle, both humane stockmanship and thoughtful facility design contribute greatly to their welfare. This chapter identifies the species-typical behaviors of cattle and recommend ways to house the animals in the best possible environment.

Cattle-typical behavior

Cattle live in herds as a primary means of protection from predators. The size of the herd is dependent upon the natural resources available. The social structure of a herd is based upon social rank relationships. Shifts in rank position are inherent in the dynamic interdependency between age, body weight and current social rank. Up to the age of about 9 years, cattle tend to gain in dominance until they occupy the best positions in the herd's social hierarchy. This is usually a short-lived dominance. In general, after the age of about 10 years, cattle show a progressive decline in status, which is paralleled by a gradual loss of body weight and body strength. Tension observed in cattle occurs more often among animals who are of similar age and size. Younger/smaller animals do not typically create problems as they are naturally submissive towards older and larger animals and avoid social conflicts with adults in the herd (Wagnon et al., 1966; Reinhardt & Reinhardt, 1975).

Cattle are typically diplomatic when they demonstrate or assert their social positions, as they use facial gestures and various body positions before resorting to overt aggression. Overt, possibly injurious, aggression is very rare in free-ranging cattle (Schloeth, 1961; Reinhardt et al., 1986). Dominant animals have privileges that are respected by their subordinate rank partners. Subordinate animals will move out of the way when a dominant partner shows the intention to get access to a resource such as water, food, comfortable resting place, or shade.

This behavior can become an issue when groups of cattle are kept in confined housing conditions, as low-ranking animals may be deprived of essential resources. Subordinate

animals may also have problems keeping an acceptable distance from their dominant partners because of spatial restrictions. This in turn can trigger threat displays or overt aggression in dominant animals; the lower ranking partners will try to yield—often without success. Thus, the lack of space can cause high social tensions and social distress, particularly in low-ranking members of the herd (Keeling, 2001; Huzzey et al., 2006; de Vries et al., 2013).

In a typical cattle herd, mother cows wean their calves when they have reached the age of about 10 months, although there is a marked gender difference. Female calves are prevented by their mothers from suckling when they are about 9 months old, but male calves are weaned by their mothers at approximately 11 months of age (Reinhardt & Reinhardt, 1981). Calving intervals are significantly shorter when cows are allowed to wean their calves naturally than when their calves are prematurely weaned by forcefully removing them from their mothers, possibly due to the intense stress caused by the separation (Reinhardt, 1982). Young bulls can start copulating with cows several months after they have been weaned naturally, although adult bulls tend to prevent them from mating before about 16 months of age. In an undisturbed setting, heifers conceive at the average age of 25 months.

Cattle develop strong cohesive relationships with other herd members, primarily within a matrilineal lineage. Mother cows prefer their progeny over unrelated calves as grooming and grazing partners. These affiliations can last for many years beyond the time when the calves have become fully mature sexually. Comparable attachments also exist between siblings. This social cohesiveness is a key component of a successful herd and is reflected in their many synchronized behaviors (i.e., grazing, resting, movement).

Being removed from familiar conspecifics is very distressing for cattle. This is particularly true when the strong mother-calf bond is forcefully broken in the process of premature weaning (von Keyserlingk, 2007); but adult cattle also show intense distress responses—such as behavioral and vocal agitation, increased heart rate, and increased cortisol output—when they are removed from other conspecifics and kept alone (Hopster & Blokhuis, 1993).

Cattle spend most of the 24-hour day at rest, either sleeping or ruminating. When they have a choice, cattle prefer lying on a relatively soft and dry surface rather than on a hard and wet one (Von Wander, 1976; Irps, 1983; Jensen et al., 1988). As such, cattle used in biomedical research should be provided with bedding to create a comfortable resting surface. Acceptable options for bedding can include straw, corn stover, old hay, soybean residue, oat hay, wheat straw, and wood chips/shavings or paper. Mixing bedding types is recommended. Sawdust or paper can be used to soak up the copious urine and fecal production, while straw or shavings provide comfortable bedding. When cleaning or rousing resting cattle, it is important to remember that typical cattle behavior involves urinating and defecating upon standing. Urination and defecation while lying down can be a sign of health issues. Availability, cost, and concern about contamination potential for experimental design are factors that may be considered when determining the bedding options.

A strong social disposition, along with general amiability, are the key characteristics that led to the domestication of cattle more than 10,000 years ago. When they feel threatened, cattle will not defend themselves, but rather run away as a group. They are usually not aggressive animals and do not shun contact with humans who are trustworthy. In fact, they are curious and will approach a friendly human.

They not only love to be groomed by other conspecifics but they equally enjoy it when a person rubs and scratches them firmly but also gently. When no grooming partner is available, cattle will scratch themselves on low branches, tree trunks, or other suitable objects to relieve itching caused by flies, parasites, or dirt.

Cattle are remarkably sensitive to prolonged exposure to direct sunlight and are prone to suffer from heat stress when they have no access to shade (Kidd, 1993). Heat stress causes cattle to exhibit changes in their behavior, physiology, and immune function; it not only creates a welfare concern but also affects reproduction (Silanikove, 2000).

Addressing cattle-typical behavior in the research lab

Cattle should always be housed in a social setting. It has been shown in heifers that they need the presence of at least one other conspecific to cope with distressing circumstances such as being moved to an unfamiliar environment (Veissier, 1992). The presence of a familiar person also buffers behavioral but not physiological stress responses to an unfamiliar environment (Rushen et al., 2001).

If a cow, heifer, or calf is to be housed individually for officially approved research-related reasons, prior housing arrangements must be made to assure that the research subject can maintain uninterrupted visual and acoustical contact with at least one nearby, familiar cow, heifer, or calf. A big mirror can distract a socially isolated heifer for a short while (Piller et al., 1999), but it is not an acceptable substitute for having a companion nearby.

Transferring cows or heifers into different groups is a source of stress and must be avoided in the research setting (Schein et al., 1955; Porzig, 1969; Arave & Albright, 1976; Dobson et al., 2001; Rousing, 2006). When cattle are removed from their familiar environment and introduced into a different one, numerous stressful and even distressful situations are created that are bound to destabilize the animals' physiological equilibrium. All of the following may occur: (a) The animal who is removed from the herd will be distressed; (b) with one herd member being removed, the old herd will have to reorganize its hierarchical structure in order to restabilize the social system—this process is bound to temporarily increase social tensions between all herd members and increase stress or distress in some individuals who lose high rank positions; (c) the introduction of the new animal into the already established social rank system of the new group will necessitate a shifting of rank positions so that the newcomer gets integrated; this process

will also lead to heightened social tension within this group and will be associated with high stress levels for the newcomer.

Cattle need sufficient space to avoid social conflicts arising from subordinate animals being unable to maintain an appropriate distance from dominant herd members, especially in situations of competition over access to food, water and comfortable resting sites. Animals of lower social rank are displaced during feeding, drinking and resting more often than higher-ranking animals (Huzzey et al., 2006). The design of a research facility that works with cattle must take the social hierarchy of a cattle herd into account. There must be enough feeding spaces, enough drinking spaces, and a comfortable resting area that is large enough so that even the lowest ranking member of the herd can access these basic resources without fear of being pushed away by higher ranking herd members. Visual barriers have a protective effect for subordinate animals during feeding time (Bouissou, 1978; Huzzey et al., 2006).

Cattle prefer somewhat cooler temperatures than most research species. Cattle housed in a laboratory setting should be kept at a thermo-neutral temperature range of about 18°C (65°F; Keown & Grant, n.d.).

Unlike at production facilities, cattle in research settings do not need extra grain and silage. Diet should be primarily a high-quality hay, provided by a reputable source and kept in a clean and dry location. Alfalfa cubes and grain-based diets should be avoided in the laboratory setting, as they do not have sufficient fibrous content to maintain proper gut health. However, it is acceptable to provide them in small quantities as dietary treats.

Prematurely removing calves from their mothers creates a distressing situation, both

for the calves and the mothers. Calves who have been naturally weaned by their mothers will show significantly lower physiological stress responses to disturbing situations than calves who have been prematurely removed from their mother in order to be artificially weaned (Lay et al., 1992).

Research facilities must take care to avoid common maladies such as lameness and leg injuries. Solid, slightly rough flooring is optimal to give cattle the best footing and sense of security. Raised flooring, while attractive because it allows for dispersal of urine and feces, should be avoided, unless it is does not move and is not slippery when wet. Adequate, deep bedding and/or the use of stall mattresses are recommended. Frequent addition of new bedding material, as well as providing regular upkeep to that bedding material, are important, as cattle produce large quantities of urine and feces (Chapinal, 2013). Care should be taken to ensure that dirty bedding does not become caked on the skin or in the hooves. Routine examination of hooves, followed by appropriate trimming and/or cleaning, will minimize hoof-related health problems.

When moving cattle, it is important to provide them with solid footing to prevent slips and reduce anxiety from walking. This can be difficult in many research facilities, as floors are designed to be easy to clean (i.e., smooth). Concurrent with problems from walking on smooth surfaces is the issue of flooring changes. Cattle can become unnerved when they encounter a change in flooring and may stop walking. Pulling them is not recommended, as this is highly stressful to the animals and can result in slips and injuries. Placing mats on the floor can be helpful. Gentle pushing from alongside the animal can also be used.

Tie stalls—where the animals are tethered to the front of the stalls by neck collars, keeping them in the stalls and preventing them from roaming the facility—do not provide proper housing conditions for cattle and must be avoided in the research setting; it hinders normal lying-down behavior and can frequently result in knee and hock inflammations (Krohn & Munksgaard, 1993). If the research objectives require tethering, then the tether must be long enough to allow the animal to stand and lie down in a species-typical manner, defecate away from bedding, and attend to other physiological needs.

Stall design must allow cattle to exhibit species-typical body movement and postures. Anderson (2001) states that cows should have a resting area that provides them the freedom to—

- » stretch the legs forward;
- » lie on one side, with unobstructed space for neck and head;
- » rest the head against one side without hindrance;
- » rest the whole body, including the tail, on the platform;
- » stand or lie without fear or pain from neck rails, partitions, or supports; and
- » rest on a clean, dry and soft bedding.

Cattle (particularly calves) will take advantage of many inanimate objects as enrichment, engaging in play behavior with them and using them to groom. Recently, commercially produced automatic articulating brushes have been introduced to provide cattle with a way to engage in this behavior. The brush remains idle and starts moving upon contact with the animal. The author has witnessed several of these brushes and they are widely used by the animals; the brush allows them to be groomed over the entire top half of their body and face. The animals wait in line for their turn to use such a brush.

Individuals working with cattle in the research setting must have a good understanding of typical cattle behavior and how to work with the animals to accomplish their goals. The fact that cattle are relatively docile animals who show little or no self-defense behaviors makes them susceptible to callous treatment in stockyards (e.g., yelling or shouting, slapping, punching, hitting with an object, tail twisting, or use of an electrical prod). Cattle are sensitive animals, who respond positively to kind and gentle interactions (Kidd, 1994).

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Pigs

Pigs

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Swine and human beings have had an important relationship since the swine's domestication, about 9,000 years ago in central Asia. The domesticated swine has been a secure food resource for many human societies. The utilization of swine in medical research dates as far back as the 16th century; however, there has been a significant increase in their use as research models over the past 50 years (Bollen et al., 2000). Swine have been used for a diverse range of studies, including cardiovascular, integumentary, and behavioral. Swine have also become a primary surgical training model for organ transplantation and other complex surgical procedures. (Smith & Swindle, 2006). They are hearty, highly intelligent animals who adapt relatively well to the laboratory environment. However, those in charge of their care must take into consideration their natural behaviors and dispositions so the animals are not stressed by species-inadequate housing and handling conditions. Stressed swine will be difficult to manage and will not provide reliable research data.

Natural environment and species-typical behaviors of pigs

Wild pigs (*Sus scrofa*) are highly adaptable and are able to live in almost any environment, but tend to be found mostly in river valleys and wooded areas. Their home range may be anywhere from 1 square mile (1.6 km^2) to up to 10 square miles (16.1 km^2), depending upon food availability, with a smaller range normally preferred (Arey & Brooke, 2006).

Wild pigs reside in small, strongly bonded social groups of three to five animals, with social rank determined by sows and their young. Younger males will often form bachelor groups, but become solitary once they reach adulthood and sexual maturity. Social rank begins very early in swine, with piglets assuming a teat order just a few days after birth, with the more dominant piglets consistently using the anterior teats for the superior milk supply. Once established, social

rank will remain stable unless new animals are introduced to the group, at which time some fighting may ensue to reestablish the hierarchy (Arey & Brooke, 2006; Bollen et. al., 2000).

Pigs are opportunistic omnivores and will spend a majority of their day rooting for food and eating many small meals throughout the day (Bollen et al., 2000). Rooting behavior is extremely important (sometimes seen as more important than the food consumption itself) and pigs will often spend time simply exploring their environment. They tend to be most active in the early morning and then again in the evening. The rest of the day is spent resting, rubbing against trees/rocks/shrubs, and wallowing in water and/or mud. These activities allow for the removal of parasites and also help the pig to keep cool and free from itchy, dry skin (Arey & Brooke, 2006).

Housing

When housing swine in the lab, it is important to remember the natural environment to which they would gravitate. The *Guide for the Care and Use of Laboratory Animals* provides size guidelines for the housing of one to several animals (National Research Council, 2011). Common sense should be taken into account, regardless of pen size guidelines, in order to ensure the pen is large enough for the animal(s) to fully turn around without bumping into a pen side, and that there is enough room to accommodate a defecation area separate from the feeding area. Solid, rough surface flooring with a substrate such as straw works well. Straw bedding reduces physical discomfort in pigs at all stages of life. Additionally, it provides a suitable opportunity for pigs to forage, promotes activity, and reduces abnormal behaviors such as apathy, stereotypies and anti-social behavior (Arey, 1993; Burbidge et al., 1994; Bolhuis et al., 2005). If however, straw is not ideal due to floor drains, a drain trap may be placed (Batchelor, 1991) or coated grated floors may be utilized. Uncoated slatted flooring is not recommended, as it does not provide good footing and pigs tend to slip. If housed on raised floors, swine should be provided with a large strong mat for resting and their hooves should be trimmed often, as they will not wear down naturally on the grates.

If possible, providing indoor/outdoor housing is an optimal way to enrich the pigs' environment, although occasional access to the outdoors is a reasonable compromise. If provided, outdoor flooring should be rough concrete and easily sanitized. Also, if swine are allowed free access to the outdoors, precautions should be taken to keep the animals from overheating or getting sunburned. Water baths, sprinklers, and sunscreens are highly recommended.

The housing area should not be barren. Swine require an enriched environment, including toys, novel objects, and foods, in order to stave off boredom. If no opportunities for rooting are provided, pigs will quickly turn to substitutes such as flooring, pen mates, and/or human caretakers. Additionally, the housing area should contain a properly placed and fixed object for the animal(s) to rub against. The author has found round, heavy-duty brushes from floor buffers to be ideal. The brushes are easily hung onto pens via heavy chain and brackets, and are strong enough to withstand larger swine heavily rubbing against them and/or rooting against the bristles.

Socialization

With the exception of intact boars, swine should be housed in pairs or groups, unless there is a scientifically supported justification for single housing approved by the veterinarian or Institutional Animal Care and Use Committee. If pigs must be housed alone, they should have access to visual, auditory, olfactory, and somatic contact with another pig to obviate physiological stress responses to social isolation (Herskin & Jensen, 2000). Side-by-side pens with open bars allow for snout touching, active play, and sleeping next to each other. Also, unless prohibited due to health or research status, when their pens are cleaned, pigs should be free to run and play together in the room. This allows not only for socialization, but also for the pigs to stretch and expend pent-up energy.
Domestic swine have a social structure similar to that of their wild counterparts and will develop strong social bonds. However, fighting may occur if new animals

are introduced or brought into an already established group (Rushen, 1990; Barnett et al., 1994; Arey & Franklin, 1995; D'Earth, 2004). Thus, it is recommended that new animals be introduced in a neutral environment in order to lessen the likelihood of fighting. If it is necessary to pair or re-pair swine often, it is best to have a separate room for the introductions. The author, along with colleagues, found that a playroom where swine were allowed to root for treats in pine shavings, as a pair or group, decreased the likelihood of aggression and facilitated relatively smooth introductions. This led to the formation of rank relationships that remained stable when the animals were moved as a group to a new stall (Casey et al., 2007).

Training

Swine are intelligent and are easily trained to cooperate with husbandry and research procedures, such as standing still for biophysical measurements (Chilcott et al., 2001), permitting physical examinations, electrocardiography, dermal dosing (Blye et al., 2006), and even nasal dosing (Brodersen et al., 2010). Regardless of the procedure, force should not be used at any time. Swine are strongly averse to force and will not only become uncooperative, but will develop fear and distrust of the handler, which may result in the animal resorting to defensive, potentially injurious aggression toward all staff. Additionally, rough handling has profound effects on the normal physiology and behavior of swine (Hemsworth & Barnett, 1991). Swine who are fearful of humans show a marked increase in corticosteroid levels both in the presence and in the absence of people (Barnett & Hemsworth, 1986). Thus, rather than force, positive reinforcement and gentle handling methods should be utilized. Techniques such as target training and clicker training can allow a handler to lead a swine into a holding cage or onto a weight scale (Pell et al., 2010; Neubauer et al., 2011), or to train a swine to cooperate for blood collection. Squeeze bottles containing juice are very useful, as they allow reward to be squeezed directly into the swine's mouth during training. Other small food items such as jelly beans, pieces of cookie, mini marshmallows, or manufactured treats also serve well as reward items. However, one must be careful not to use too many treats, as the animal may start to refuse to eat the standard diet.

Should restraint be necessary for a project, swine should never be tethered, as they become highly stressed (Barnett et al., 1985; Schouten et al., 1991) and will often vocalize from fear. Pig boards are useful for short-term restraint of larger swine, but for longer periods of time, a sling is recommended. Swine often learn quickly to walk into the sling, but may also be trained to be placed by lifting (Williams & Watson, 2003). Over just a few sessions, swine become quite comfortable and will remain relaxed—or even fall asleep—for several hours (Panepinto et al., 1983; Grandin et al., 1989).

Enrichment

Due to their high level of intelligence and behavioral needs, swine require an enriched environment. If left in a barren environment, swine may resort to stereotypical behaviors such as pacing, or may begin to chew on enclosures, feed/water bowls, or even pen mates. Thus, it is very important to provide environmental enrichment for swine, with rooting activities being the most important of all, as rooting is a behavioral need that is performed regardless of feeding level or nutritive feedback (Beattie & O'Connell, 2002).

Swine have an innate need to root and will do so on any and every surface within their reach. Straw/hay with hidden treats such as jelly beans, pieces/chunks of apple, whole apples (pigs LOVE apples!), manufactured treats, etc. allow swine to express this important species-typical behavior. Swine will also root through straw/hay when no treats are present (Fraser et al., 1991), making this activity an option even in those cases where novel food items may be restricted due to research protocols. In such cases, scattering food on the floor, or on the straw bedding, is an excellent alternative to bowls, as this allows the pigs to perform their natural rooting behavior without added confounds (Beale et al., 2007). In cases of raised floors and/or drains where straw/hay may be problematic for sanitation, it is recommended to provide opportunities for the expression of this activity in another separate area specifically dedicated for the purpose (Casey et al., 2007). Other enrichment devices such as balls, Kongs, and thick-walled cardboard tubes are recommended for indoor pens with drains.

Filling toys such as Kongs with pieces of fruit or other treats adds more incentive for the pig to use them. Boomer Balls (of appropriate size) filled partially with juice and then frozen can provide a challenge and a great deal of fun for all swine.

Pigs also enjoy playing with hanging items (Young et al., 1994). Tug toys, pieces of fire hose, Jolly Apples and other hanging items—such as forage balls baited with treats (Huntsberry et al., 2008)—are well received by swine of all sizes. The author has found that short sections of hanging chain seem to always be of interest to swine, although they prefer to play with soft pliable objects when given a choice (Grandin, 1988). Pieces of thin cloth such as bed sheets can make great tug toys for swine (Grandin & Curtis, 1984). They are readily tied to pens and are easy to change/replace when they become soiled—a necessary practice since swine will ignore or avoid any enrichment item that

becomes soiled with fecal material (Grandin, 1988). Any enrichment item that becomes soiled must be cleaned or removed from the pen in a timely fashion.

Human interaction

Swine are very social and affectionate animals, making it easy for their caregivers to forge a positive relationship with them. Regular, positive human interaction not only helps pigs become more comfortable in their surroundings (Geers et al., 1995) and overcome fear and stress responses to people (Gonyou et al., 1986), but may also serve as an appropriate enrichment for the singly housed swine. The simple act of squatting down, speaking softly, and providing a good snout rub can allow for a bond to form between the pig and the person almost immediately. Additionally, interactive enrichment activities help to make the swine more comfortable in their surroundings. Playing "sprinkler" with a garden hose (being careful not to get the pig overly wet!) or tossing cut-up apples into a water bowl for bobbing are great activities for all staff in the institution to share with the swine. The more positive interaction pigs receive, the more likely they will be calm and cooperative during husbandry and research procedures.

Final thoughts

The late Maurice Sendak centered one of his final books upon an orphaned piglet: Bumble-Ardy, who's only wish is to have a birthday party. In an interview with Avi Steinberg of the *Paris Review*, in 2011, when asked why he chose a pig, he said, "I've always loved pigs: the shape of them, the look of them, and the fact that they are so intelligent. ... The prospect of drawing pigs was something I could look forward to, and I needed something to look forward to."

Working with pigs is indeed something to look forward to. It is highly rewarding, and, at times, is a great deal of fun. A naturally cheerful species, pigs are capable of generating great joy, and often give more than they receive. The author hopes the tips provided in this chapter will bring many pigs, and many people, more fulfilling days in the laboratory.

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Sheep

Sheep

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Sheep (*Ovis aries*) have been domesticated for thousands of years. They are used in biomedical research in the United States in studies ranging from investigations of asthma and respiratory disease to development of novel cardiac interventions (Scheerlinck et al., 2008). This chapter will discuss some characteristics of sheep that are important to consider in the research laboratory, and will provide recommendations for refining research protocols involving sheep.

Species-typical characteristics of sheep

Kilgour (1976), the father of modern sheep ethology, described the sheep as a "defenseless, vigilant, tight-flocking, visual, wool-covered ruminant ... displaying a follower-type dam-offspring relationship, with strong imitation between young and old."

These characteristics are fundamental to describing and understanding the normal behavior of sheep in research facilities. The social nature of sheep is their most notable characteristic in terms of understanding and considering their behavior, and the instinct of sheep to flock tightly and to maintain a visual link with other sheep is perhaps the most important consideration for housing sheep in the laboratory.

Sheep establish well-defined relationships and strong bonds within the group; they can remember the faces of conspecifics for up to 2 years (Kendrick et al., 1996). Individual sheep tend to synchronize their activities with other sheep in the flock, as they walk and run together, follow one another, graze together, and lie down or ruminate together (Hutson, 2007). When separated from the group, individuals run towards the other sheep even when the path is obstructed by a handler or dog (Kilgour, 1977). Lambs instinctively follow their mothers. This following behavior remains in adulthood and can be utilized to handle sheep efficiently without undue stress.

Sheep are primarily grazers with absent maxillary incisors, allowing grazing close to the ground; however, sheep will also browse when they have access to low branches or shrubs. Although flocks move together in a leader-follower pattern, high behavioral synchronization for other activities, like grazing, is not as apparent as in cattle. Sheep are not territorial, but do utilize a home range, which may be shared with other groups. When a home range is shared, sheep recognize members of their own group and avoid animals from other groups (Dwyer, 2008). Ewes and juvenile animals form matrilineal groups, and rams form small bachelor groups. Dominance between rams

may be established by physical contact in the form of nudging and head butting, especially in the mating season. Submissive individuals lower and twist their head sideways, and avoid the dominant animal in the future (Ekesbo, 2011). In contrast, dominance is not apparent among similar-age ewes. Outside of competitive situations, agonistic behaviors have not been reported among ewes, even in studies in which flocks from different origins were mixed (Dwyer, 2008).

As a “defenseless” prey species, the sheep’s main anti-predator strategies are flocking and flight (Dwyer, 2008). Sheep are disturbed easily, and can be frightened by sudden sounds or fast movements, especially in unknown or confined areas, an important consideration for handling sheep in the laboratory. If one animal adopts an “alarm posture”—with head raised rigidly, while using slow, tense steps—this rapidly alerts others in the group (Geist, 1971). With a visual field of 280°, sheep are able to maintain awareness of potential predators and spatial relationships with others in the flock (Hutson, 2007). In response to perceived danger, sheep will readily flee. In the research setting, all handlers should be aware of this instinct, as the wild flight of panic can cause harm to the sheep and/or the handlers (Ekesbo, 2011).

Ensuring the welfare of sheep

Since the Brambell Report was first published in 1965 in response to concern for farm animal welfare in the United Kingdom, the “Five Freedoms” in that report have become the minimum standards of care for farm animals (Brambell Committee, 1965). The enumerated freedoms are as follows:

1. Freedom from thirst, hunger and malnutrition—by ready access to fresh water and a diet to maintain full health and vigor.
2. Freedom from thermal or physical distress—by providing an appropriate environment, including shelter and a comfortable resting area.

3. Freedom from pain, injury and disease—by prevention or by rapid diagnosis and treatment.
4. Freedom to display most normal patterns of behavior—by providing sufficient space, proper facilities, and company of the animals' own kind.
5. Freedom from fear and distress—by ensuring conditions and treatment to avoid mental suffering.

Although the initial intent of the Brambell Report was to improve the welfare of animals on the farm, these principles apply to sheep in a research setting, as well.

Freedom from thirst, hunger and malnutrition: An adult sheep may drink up to 6 liters per day, so continuous access to fresh, drinkable water is essential. Roughage is particularly important in ruminants such as sheep. The major component of the diet for sheep should be bulky food with a high fiber content. Diets lacking in fiber are associated with increases in abnormal oral behaviors in sheep such as mouthing bars, chewing chains, mandibulation (licking lips and mouthing air), and repetitive licking (Done-Currie et al., 1984; Mardsen & Wood-Gush, 1986; Cooper et al., 1994; Yurtman et al., 2002). Hay or an increased fiber diet reduces these oral stereotypies (Done-Currie et al., 1984), and adequate roughage reduces the incidence of maladaptive behaviors such as wool-biting (Vasseur et al., 2006).

Sheep on pasture may graze up to 11 hours and ruminate up to 8 hours per day, and even animals whose nutritional needs are met by a concentrated feed ration still exhibit food-searching behaviors (Ekesbo, 2011). Hanging hay in suspended baskets or nets or providing fresh browse are relatively inexpensive and easy ways to provide opportunities for indoor-housed animals to engage in feeding behavior for longer periods, to help satisfy the biological need for grazing.

Freedom from thermal or physical distress: Sheep in the wild are one of the most successful animal species, with a nearly global distribution over a wide range of terrains and climates (Dwyer, 2008). Outdoor housing on pasture provides sheep with the most opportunity to express species-typical behaviors. Animals housed exclusively outdoors must be provided with shelters to withstand weather extremes (National Research Council, 2011), and sheep will use such shelters particularly as protection against strong wind. Sheep are extremely well-adapted to cold, with a lower critical temperature as low as 0°C in fully fleeced adult animals (Terrill & Slee, 1991). Protection from predation, particularly domestic dogs, is also important in outdoor housing situations (Dwyer, 2008).

When an institution has not been able to provide housing for the duration of a research study, the author has utilized a local farm in the area to house animals who needed to be followed for an extended period but required only minimal experimental sampling. In the author's experience, transporting the animals to the farm resulted in minimal stress and improved their well-being for the 6–12 months they were able to be outdoors, compared to the indoor pens available in the medical center setting. Sheep may be housed in outdoor groups even during studies. The sheep in the photo to the right underwent cardiac surgery and were housed in an indoor pen for recovery. Afterwards, the animals were moved to a large, outdoor pen where they could engage in species-typical behaviors for the remainder of the study.

In contrast to outdoor housing, the indoor environment in research is typically well controlled within a narrow range of environmental parameters. For indoor housing, the *Guide for the Care and Use of Laboratory Animals* states that sheep should be maintained at a dry-bulb temperature of 16–27°C (61–81°F; National Research Council, 2011). Animals kept indoors should be sheared to prevent heat stress. Flooring should be solid or slatted, with a slip-resistant surface. Routine foot checks should be part of the program of veterinary care, especially for animals housed indoors. The floor surface should be such that it provides some wear on the hoof to minimize the requirement for hoof trimming, but avoids excessive hoof wear. In indoor facilities where floors are slippery and replacement is not an option, rubber matting can be used to improve sheep comfort. In the author's experience, providing sheep with firm footing greatly increases their compliance with light restraint for minimally invasive procedures.

Hay and straw are highly recommended bedding options for sheep, not only on farms, but also in the research setting. Wood chips, corn cobs, and paper products have been used in indoor facilities, and these alternative bedding options may have advantages in terms of moisture absorption and cleaning needs. However, straw and hay provide the animal with more opportunity for foraging, so should be used whenever possible.

Regulations regarding minimum cage sizes for sheep have been promulgated, but animals must also be provided with adequate space for normal ambulation. Pen sizes should be large enough, or cleaned frequently enough, so that all members of the group may simultaneously lie in clean, dry areas of the pen.

Freedom from pain, injury and disease: Health, i.e., normal biologic function, is one of the most basic aspects of welfare. Like other research animals, sheep must be provided with adequate veterinary care, including a routine preventive health program of vaccinations, anthelmintics, and ectoparasite control; frequent observations by trained personnel to monitor for health problems (including the need for hoof trimming); and access to an experienced veterinarian for prompt diagnosis and treatment of health problems.

Prey animals, in general, instinctively hide signs of pain or disease, and sheep in particular have been described as "stoical" or "physically tough" (Webster, 1994). With the exception of lambs separated from their mothers, sheep are rarely vocal in response to stressors (Dwyer & Lawrence, 2008). However, sheep do feel pain and are subject to the same behavioral and physiologic consequences of pain as other research animals who may display signs of pain more readily. Unlike cattle, sheep do not vocalize in response to painful procedures (Hutson, 2007), so absence of vocalization does not indicate the animal is not experiencing pain. In sheep, signs of pain include more subtle changes in appetite or facial expression, reduced cud chewing, adoption of a rigid stance with lowered head, bruxism (teeth grinding), and withdrawal from the group. In the absence of specific evidence to the contrary, any procedure expected to cause pain or distress in humans should be considered painful or distressing in sheep (Interagency Research Animal Committee, 2011), and appropriate analgesics must be given under the direction of a veterinarian.

Many sheep used in research are not purpose-bred and are acquired from farms where they may be subject to standard agricultural practices. In some cases, these practices include dehorning, castration, and/or tail-docking without anesthesia or analgesia (Federation of Animal Science Societies, 2010). Responsible research institutions should request that these procedures not be done, unless required by the research, and then, only with appropriate anesthesia and analgesia.

Freedom to display most normal patterns of behavior: As previously discussed, gregariousness is a key behavioral characteristic of sheep. Sheep also display leadership, a social behavior in which one animal initiates a movement and is subsequently followed by the other members of the group (Nowak et al., 2008). Failure to provide appropriate social companionship to sheep is associated with a myriad of endocrine, hematological, biochemical, and behavioral alterations. Isolation reduces growth rate and decreases feeding time (Abdel-Rhaman, 2000). Isolation persistently elevates cortisol level and heart rate (Cockram et al., 1994), and actually activates the endocrine stress response (e.g., hypothalamic-pituitary-adrenal axis) to a greater degree than does handling or restraint (Baldock & Sibly, 1990).

Although social companionship is required for adequate welfare, aggression occurs when social groups are mixed or stocking densities become so high as to restrict access to resources (Arnold & Maller, 1974; Done-Currie et al., 1984). In stable groups, dominance hierarchies are well defined and maintained through nonaggressive behaviors (Guilhem et al., 2000), so the occurrence of aggressive behaviors can indicate some form of management and/or husbandry-related stress within the group.

In many species, demonstration of stereotypic behavior is a hallmark of poor welfare, but sheep may be less likely than other species to display maladaptive behaviors (Houpt, 1987; Lawrence & Rushen, 1993). However, individually housed sheep demonstrate stereotypical oral and locomotor behaviors (Done-Currie et al., 1984; Marsden & Wood-Gush, 1986; Yurtman et al., 2002). Improper housing may incite stereotypical behaviors. For example, wool-pulling only occurs in indoor-housed sheep, and the behavior is obviated when sheep are turned out in pastures (Dwyer, 2008) or the fiber content of the diet is increased (Vasseur et al., 2006). As stated above, sheep typically graze for up to 11 hours a day (Lynch et al., 1992), so providing grazing opportunities is important to foster the expression of species-typical behavior. In indoor facilities, equine hay nets provided as "puzzle feeders" can be a good substitute for grazing (Atkins et al., 2007). In the author's experience, these nets not only increase foraging time, but also reduce hay wasting by the animals.

Freedom from fear and distress: Sheep are particularly apprehensive when subject to restraint and handling. This fear induces physiological changes that can confound research data. For example, stressed sheep have a reduced lymphocyte blastogenic response when challenged with certain mitogens (Minton et al., 1992; Minton et al., 1995). Restraint, confinement, and transport can block or delay leutinizing hormone (LH) secretion, resulting in suppression of follicular growth and reduction in estradiol (Rasmussen & Malven, 1983; Dobson & Smith, 1995). Isolation is also a potent fear inducer in sheep, and animals do not adapt to isolation even when subjected to it repeatedly (Niezgoda et al., 1987).

Positive human contact can reduce fearfulness and subsequently the stress of handling procedures (Dwyer & Lawrence, 2008). Sheep may form a bond with a caretaker that allows the animal to develop coping strategies to handling and experimental manipulations (Wolfle, 1996).

Refining husbandry and research procedures for sheep

Although sheep have specific nutritional, physiologic, and social needs, their domestication has made them an adaptable, placid laboratory animal species. Catering to the sheep's unique needs will not only improve their welfare, but as previously discussed, will ease experimental manipulations and improve research outcomes.

Relatively little research on handling methods has been done with sheep, but the same broad principles that apply to proper cattle handling are likely applicable to handling sheep, as well. For example, handlers should avoid the use of fearful stimuli and punishment, and instead choose positive reinforcements. They should understand sheep behavior and take advantage of sheep-typical leading/following, and form positive relationships with the animals. In the United States, the Animal Welfare Act requires that research facilities provide adequate training (Animal Welfare Act, 2012). This training should include basic instructions in animal behavior.

Following arrival at the institution, sheep should be given an acclimation period of at least 1 week before any aversive or negative experiences. Importantly for research, this period allows the animal's immunologic and physiologic stress responses triggered by shipping and transport to return to baseline. During this time, sheep can adapt to the new housing situation and husbandry routine. This time period may be especially crucial for animals transferred from outdoor pastures to indoor housing, as this transition is particularly disorienting and distressing for sheep (Done-Currie et al., 1984; Fordham et al., 1991). Although a 1-week period is customary, it may take up to 4 weeks before cortisol levels return to baseline after sheep are transported from pasture to indoor housing (McNatty & Young, 1973). Transport has also been associated with unwillingness to eat new foods (Dwyer, 2008), so animals must be observed closely for adequate food and water consumption. Consistency in husbandry and handling routine and stable group composition are essential for adaptation in the sheep (Fraser, 1995), so staff should avoid changing the composition of an established group of sheep. The animals develop expectations based on their previous experiences, and deviation from the expected routine reportedly causes increased heart rate and agitation (Greiveldinger et al., 2007).

The acclimation period also provides time for staff to gain the trust of the animals, so that future manipulations may be less stressful. Since sheep can distinguish visually between familiar and unfamiliar humans (Kendrick et al., 2001), an acclimation period gives the animals an opportunity to become familiar with their caretakers. Gentling, or stroking the head of the sheep, talking quietly, and hand-feeding results in familiarization with staff and habituation with routine husbandry procedures. Well-familiarized sheep approach humans more readily, have shorter flight distances, and lower heart rates (Hargreaves & Hutson, 1990; Mateo et al., 1991). These sheep will typically accept a potentially stressful situation, such as blood collection, more readily (Kilgour, 1987). Over the course of the acclimation period, the caretakers will become familiar with the animals and sensitive to subtle behavioral changes that may indicate pain or distress during future research procedures.

The sheep's innate following behavior should be exploited to provide a more positive handling experience for both the animal and the researcher or animal care technician. Encouraging this leader-follower behavior can facilitate routine procedures such as weighing and veterinary examinations (Hutson, 2007). In most cases, the animals will proceed as a group to a target location away from the handler simply as a response to the handler encroaching on the leader's flight distance. The use of fear stimuli, such as loud noises or rapid movements, unnecessarily frightens the animals, and activating the animals' flight instinct will make them less compliant and less likely to do what is expected of them.

The single most important welfare aspect of sheep is the biologically inherent need for social companionship. It is imperative to maintain stable social groups in research facilities. Companions assist the individual sheep in coping with disturbing situations, and they buffer the stress and fear response experienced in husbandry and research procedures (Federation of Animal Science Societies, 2010; Gonzáleza et al., 2013; Porter et al., 1995). Prolonged isolation may be associated with reductions in food and water intake and (as noted above) activation of the animal's endocrine stress response (Apple et al., 1993; Carbajal & Orihuela, 2001). When animals must be singly housed, slatted or chain-link fences should be used to allow for visual and protected physical contact (Baldock & Sibly, 1990). When sheep are isolated for use in metabolism studies, the stress effect of this isolation must be considered, as individual housing may increase the sheep's metabolism up to 15% (Van Adrichem & Vogt, 1993). The presence of a single companion is sufficient to mitigate the physiological and behavioral effects of isolation (Carbajal & Orihuela, 2001). Mirrored panels or familiar sheep-face pictures may be used when no conspecifics are within view to mitigate anxiety and avoid

panic responses to isolation (da Costa et al., 2004; McLean, 2004; Parrott et al., 1988). In the author's experience, intermittent supervised periods of free contact with conspecifics serve as a social facilitator to improve appetite when animals must be singly housed for experimental purposes. Such periods of social relief foster the well-being of the lonely sheep.

When caretakers have formed a positive relationship with the individual sheep, the human-animal bond can serve as a substitute for conspecific social contact. For example, lambs vocalized and moved less when in the presence of a shepherd than when isolated (Boivin et al., 1997). However, research facilities should facilitate constant social companionship, with strategies such as basing per diems on pairs of animals or creating a pen charge for two or more animals. These strategies allow the housing of companion animals not being actively used for research at no additional cost to the researcher.

Stable social relationships are essential; changing the composition of established groups by introducing or removing sheep may be a significant source of stress resulting from disrupted social relationships and rank-determining aggressive interactions (Sevi et al., 2001). When neonates or pre-weanlings are needed for research, every effort should be made to keep the lambs with their mothers until the age of natural weaning. Separation of mothers from offspring is one of the few occasions that result in vocalization in sheep, and lambs prematurely separated exhibit behavioral and physiologic responses indicative of stress, including increased cortisol levels and impaired immune responses (Moberg & Wood, 1981; Napolitano et al., 1995; Price & Thos, 1980).

Staff should be trained in and actively employ positive reinforcement training when handling sheep. Food rewards like barley or grain are readily accepted by sheep, and although some consider sheep as incapable of learning, sheep can be easily conditioned (Hutson, 2007). For example, in the author's facility, sheep have been taught to drink from a syringe, stand still for venipuncture, and shift from one pen to another during cleaning. Sheep reportedly remember distressful experiences for at least 12 weeks (Rushen, 1986) and up to 1 year (Hutson, 1985), but also will return to places where they were manually restrained if the experience was positive (Grandin, 1989). Using food rewards not only reduces the stress response to handling, but also improves the speed of handling, and these positive effects on handling are maintained for at least a year (Hutson, 1985). Training sheep to participate in procedures provides the animal with an element of control, which has been shown to affect the sheep's emotional response to a disturbing situation (Greiveldinger et al., 2007).

Summary

Taking into account the unique behavioral needs of sheep will improve animal welfare and research outcomes. The management of sheep in research institutions must meet the following requirements:

- Sheep are housed in stable social groups.
- Sheep are provided appropriate housing with bedding and bulky food.
- Staff members are trained in sheep behavior.
- Gentle familiarization is employed for new animals and positive reinforcement training is used with all animals to promote cooperation during potentially aversive experimental procedures.

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Dogs

Dogs

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Dogs have a special status in our culture, where they are known as "man's best friend" or "constant protector" and often viewed as members of the family. Americans spend billions of dollars every year on their companion dogs (Associated Press, 2014). This places raised expectations on the laboratory animal community to not only care for the basic necessities of laboratory dogs, but also address their social and emotional well-being. Some of these expectations are described in the *Guide for the Care and Use of Laboratory Animals,* which addresses the issues of social housing and human interaction (National Research Council, 2011).

The importance of increased awareness of social and behavioral needs

Domestic dogs are highly social animals, relying on human or conspecific interactions to fulfill their social requirements. Multiple reports have noted that social isolation is a significant stressor for dogs (e.g., Wolfe, 1992). Within the laboratory setting, there are many opportunities to provide for their social needs, as will be discussed below.

It should be the norm to house dogs with conspecifics (Overall & Dyer, 2005) and any solitary housing should be considered the exception, subject to regular reevaluation by the attending veterinarian and/or the Institutional Animal Care and Use Committee. Behavioral and personality assessments should be done before pairs or groups are formed and care must be taken to house animals accordingly. While pair-housed dogs are more common in the laboratory, larger groups can be formed if sufficient space is available and study parameters permit it (Field & Jackson, 2007).

If social housing is not possible, housing should allow for visual access to other canines. Some facilities have created play rooms or play areas, where animals are allowed to exercise and socialize (Loveridge, 1994; Evans et al., 1999; Andrews-Kelly, 2010; Shulder & Ogbin, 2010). The play areas can include a number of enrichment devices such as platforms and ramps (reminiscent of canine agility courses) and, if access to outside is prohibited, turf flooring made to resemble grass (Hubrecht, 2002). Enrichment may also involve tapping into canines' innate abilities—exploring the environment through scent or puzzle solving are two activities that engage and promote exercise and use of space (Haug, 2006). In the author's experience, having a structured schedule for human interaction and positive reinforcement training can work to both improve welfare and socialization and improve efficiency in husbandry. Training a room full of long-term canines in basic commands such as sit, come and leash walking can speed up the performance of husbandry practices and make them less stressful for the animals. In such an environment, exercise time involves obedience training and removal/return to housing is voluntary.

Even when social housing and group play facilities are available, daily human interaction is an important part of the daily regimen for dogs in a laboratory. The USDA stresses access to at least one enrichment device, as well as human interaction and social housing, as the rule rather than the exception (Overall & Dyer, 2005). These guidelines should be considered as starting points to a more facility-based and inventive housing program.

Mimicking a natural setting for animals in laboratories is encouraged. In broad terms, for domestic dogs, the natural setting is social living with daily human interaction, training, and novel enrichment devices (Wolfle, 1992). This allows for flexibility, and institutions often take advantage of this flexibility to create a plan that fits within their own constraints and staffing allowances.

Because of their ability to be trained, it is possible to modify behaviors of dogs in the laboratory to minimize the stress of handling or of minor research procedures (Trussell et al., 1999; Hubrecht, 2002; Roddis, 2005; Hussain et al., 2006; Tabers et al., 2009; Savastano, 2013). Positive reinforcement training is the preferred method (Laule, 1999). This can lead to a trust bond between technician and canine that will lead to easier daily activities. Socialization and basic training at the supplier have a considerable impact on how laboratory canines will react to handling during research and husbandry practices (Fox, 1975; Freedman et al., 1991; Trussell et al., 1999). Institutions should work with suppliers to ensure any canines are socialized to both other canines and humans.

Enrichment devices must be of a quality and construction that make them safe to leave with unsupervised dogs, and must be cleaned/checked daily for wear and tear. These can include toys used with food incentives (e.g., Kong toys), puzzle toys to create complexity for daily rations (including toys with hidden compartments for treats, or buttons and switches to obtain treats), or toys to promote chewing (Nylabone as an example, though care should be taken that no piece of the toy can be chewed off and swallowed). Toys with fleece, plush or rope could be ingested if the dog is left alone and should only be provided with supervision. If socially housed, care must be taken with high-value toys so that a fight does not occur between dogs. Multiple toys should be available in social housing situations. Separation may be necessary for food-based enrichment (Overall & Dyer, 2005).

Physical structure of housing space

There are many variations for the physical structure of the housing space for laboratory canines. The *Guide* states the space should be, at minimum, sufficient for full range of movement while standing, turning and lying down, using body weight as a guideline (0.74 to over 2.4 m^2 floor area, depending on the size of the dog). While the space guidelines can be met with caging for smaller dogs, it is always preferable to use much larger runs for all dog sizes (National Research Council, 2011).

Flooring can be a variety of substrates: slate flooring with drains beneath, solid flooring (usually concrete), sawdust, or access to the outdoors. Solid flooring with elevated rest areas and a separate space for elimination is the ideal option. Wire or slatted flooring can create pressure sores on the pads of dogs' feet (Field & Jackson, 2007). This type of flooring can also entrap toes and should be avoided. This issue can be partially addressed

with resting pads, frequent exercise out of the enclosure, and sawdust used sparingly for moisture control. Regardless of flooring type, it is important to maintain a clean and dry environment. Housing space should also be large enough to allow the dogs to avoid areas that have been urinated or defecated in. This is a particularly important point when dogs are socially housed.

When possible, elevated resting surfaces should be used. Elevation has multiple benefits, as it adds complexity to the housing space and helps keep the resting surfaces clean and dry. When constructing raised platforms, care should be taken to ensure that the platforms and areas underneath them can be sanitized. The platforms should be large enough to accomodate the size and number of dogs who might use them (Anonymous, 2013b; Hubrecht, 1993).

Diet and husbandry care

Most laboratory canines are fed a dry kibble diet that is meat based and measured according to size, age and physical activity (Hubrecht, 1995). This can be fed from a bowl on the ground, or a feeder attached to the cage or run door to maintain cleanliness. It is preferable to keep food and water bowls off the floor, to prevent soiling and accidental spillage. When dogs are housed in groups, care must be taken to ensure that all are eating an appropriate amount. If aggression over food is noticed, then group-housed dogs should be separated for feeding. Treats must be approved by veterinary and laboratory staff to make sure they do not affect study goals or impact the health of the dog. Small treats for training or insertion in puzzle toys are often used.

Water can be provided either in a bowl or Lixit attached to a constant water source. While the Lixit is a convenient way to prevent contamination of the water supply, the bowl allows for a more natural drinking motion. If Lixits are used, care should be taken to ensure there are enough to support all dogs in the enclosure and that all dogs are drinking enough to be properly hydrated. The Lixits must be checked daily to make sure they are in working order.

Husbandry care includes cleaning of enclosures, human interaction and socialization, positive reinforcement training, nail trimmings (monthly, or as needed), and daily health checks. Positive interactions with the dogs are paramount to thorough health checks, as visual and tactile observations lead to a more thorough inspection. Technicians should be well versed in the common signs of distress and pain in canines (National Research Council, 1994). It is a common practice to spray down housing enclosures for daily cleaning. If this is the practice, the occupants must be taken out of the enclosure and the enclosure should be allowed to dry before they are returned. Further, it is not uncommon for dogs to urinate or defecate in a newly cleaned enclosure. Care staff should be aware of this habit so that they can remove the fecal matter.

Noise levels

The noise levels in kennels can reach 100 decibels. This level can be damaging to human and canine hearing (Hubrecht, 1995). Some facilities try to alleviate this by playing soothing music or using white noise in the kennel, which may decrease barking and agitation (Kilcullen-Steiner & Mitchell, 2001; Wells et al., 2002). Debarking should not be considered an option to control noise levels, as it can have significant adverse consequences (i.e., swelling, bleeding, and infection) with little gain (bark is only muffled, not removed) ("Alternatives to Debarking," 2014).

Retirement and rehoming of laboratory canines

It has previously been considered an extremely difficult feat to rehome laboratory canines, especially purpose-bred hounds and beagles. While laboratory canines do necessitate a unique degree of care and ability, adoption is increasingly viewed as an acceptable alternative if euthanasia is not necessary for the studies conducted (Simons, 2014). Many facilities have in-house adoption programs that release canines primarily to staff members, many of whom form attachments during their work with a particular dog (Anonymous, 2013c). There are often terms associated with such adoptions, including the dogs passing behavioral and medical screenings and having minimal surgical interventions during their studies. For facilities with a large number of canines being rehomed (or with a particular study group that will be available) it is advised to have a special socialization and introduction program before the dogs' release to increase the likelihood of adaptation to living in a house and outdoor setting (Evans et al., 1999; Burgess et al., 2010.) Some rehoming groups will work with the dogs on socialization and preparation for life in a home.

There are also some rescue groups who specialize in rehoming the most common laboratory breed, the beagle. For example, one of the largest and longstanding groups, the Beagle Rescue League, operates out of New Jersey and will work with laboratories to ensure proper socialization and successful home placement. If an outside group is used, research should be done into the group's success in providing smooth transitions and successful placements, as well as the group's history and possible political and public agendas. If facilities wish to remain anonymous or specify any other terms, these should be clearly spelled out in a written agreement.

Conclusion

A compassionate approach to laboratory canine housing and care can lead to better research and better animal welfare. Dry, comfortable and spacious housing with adequate food and cleanliness are the basis from which to build a comprehensive care program for canines. This should include attention to their social, behavioral and training needs. This also includes encouraging rehoming after research when possible.

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Cats

Cats

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Implementation of the "Three Rs" (replacement, reduction and refinement) should be considered whenever animals are used in biomedical research (Russell & Burch, 1959). Refinement applies both to experimental procedures and to the way animals are housed and looked after. Housing conditions have a major impact on animal welfare so they should be well regulated to the highest standards. This is especially relevant for domestic cats (*Felis silvestris catus*), who cannot usually be taken out of their enclosures for walks in the way that dogs can.

Keeping cats in an enriched, stimulating environment that encourages a wide range of normal behaviors—and providing them with ongoing positive interactions with people—will result in enhanced health and welfare. In addition, when these cats are no longer required for research and are rehomed, they will be more likely to adapt successfully to their new environment (DiGangi & Levy, 2006).

Species-typical characteristics of cats

In order to understand and appreciate the species-typical characteristics of cats, knowledge of their evolutionary history is helpful. The domestic cat is descended from the North African wildcat (*Felis silvestris libyca*), a largely solitary-living, territorial species. This carnivorous predator lives and hunts alone; it is also the prey of larger carnivores. The species can be described as semi-arboreal, spending much of the day hiding in bushes and trees. Members of the species are active primarily at night, and come into direct contact with conspecifics mainly for mating.

Studies of feral cats, defined as free-living domestic cats with limited or no contact with humans, are also informative. Feral cats also hunt alone. But while they may

live singly, when there are sufficient food sources they often form groups (colonies) of mainly female relatives (Macdonald et al., 1987; Macdonald et al., 2000). Female feral cats may cooperate in the rearing of kittens (Macdonald et al., 2000), especially if they are related (e.g., mother-daughter, sister-sister). Cats can form strong social bonds with other individuals ("preferred affiliates," Curtis et al., 2003; Crowell-Davis et al., 2004), particularly if they are related: e.g., between kittens in the same litter and between kittens and their mother. They may also form close bonds with unrelated but familiar cats; this is likely to develop if they have been together from a young age. Cats who are members of the same group are often in tactile contact, exhibiting behaviors such as allorubbing (rubbing their bodies against each other), allogrooming (grooming each other), touching noses (a greeting response), and resting together (Curtis et al., 2003; Crowell-Davis et al., 2004; Rochlitz, 2009).

The wildcat relies primarily on olfactory communication, which is particularly well adapted to a solitary lifestyle, as it is long-lasting, individual, specific, and effective over long distances. Like the wildcat, both feral and nonferal domestic cats have an excellent sense of smell and rely primarily on olfactory communication. This consists of the deposition of scent (scent marking) mainly by urine spraying, claw scratching, and tactile rubbing (against objects or other cats), and less commonly by elimination (urination and defecation). A cat's nasal epithelium is dense with olfactory receptors, and there is a vomeronasal organ behind the upper incisors that is used in the "flehmen" response (Bradshaw et al., 2012). This response, where the upper lip is raised and air inhaled, is seen primarily in reaction to odors from other cats and is presumed to gather social information.

In addition to olfactory communication, cats use visual (i.e., posture, tail position, and facial expression) and vocal communication. Interestingly, they are more vocal towards humans than towards other cats (Brown & Bradshaw, 2014). It is thought that cats lack the facial musculature typical of social species (van den Bos, 1998) and do not have as large a behavioral repertoire for visual communication as, for example, the highly social, group-living dog. Nevertheless, while acknowledging that some signs may be subtle, much can be learned about a cat from careful observation.

Addressing the species-typical characteristics of cats in the research laboratory

This chapter will describe ways of addressing the species-typical characteristics of cats in research institutions, with the aim of meeting their needs and ensuring their good welfare. Much of this information is derived from reviews on environmental enrichment for domestic cats in the home, in catteries or in shelters (e.g., Rochlitz, 1999, 2000a, 2005; Ellis, 2009; Herron & Buffington, 2010; Ellis et al., 2013: Stella & Buffington, 2014), as well as in laboratories (e.g., Rochlitz, 1999, 2000a, 2000b; Overall & Dyer, 2005; McCune et al., 2014). The five main elements to the design of cat housing in research laboratories (adapted from Bloomsmith et al., 1991) are as follows:

1. Physical: size of enclosure, internal structure and complexity
2. Social: socialization—contact with conspecifics and humans
3. Sensory: olfaction and marking behaviors, visual and auditory stimuli, surrounding area within the cat's sensory range
4. Occupational: opportunities to explore the environment, exercise and play
5. Nutritional: provision of food and water, toileting (elimination)

Physical

Having evolved from a solitary-living species, cats have not been subject to selection pressures to develop a wide range of social communication behaviors or formal group structures such as strict dominance hierarchies. In addition, unlike the dog, they lack mechanisms to convey appeasement or reconciliation after conflicts (van den Bos, 1998; Casey & Bradshaw, 2007). The main way they avoid conflict is by avoiding each other. Studies have shown that a distance of 1 to 3 meters between cats is required to reduce stress and maintain harmony (Kessler & Turner, 1999; Barry & Crowell-Davis, 1999). If cats are unable to establish sufficient distance, enforced proximity may lead to a notable reduction in activity and increased stress, possibly resulting in overt aggression.

The minimum space requirements for the housing of cats in laboratories in Europe can be found in Appendix A of the European Convention ETS 123 (Council of Europe, 2006) and are presented in the table on the following page. Enclosures should be "walk-in" (2 m high or more) to allow caretakers to enter, as this makes it easier to interact with the cats in a normal manner and conduct maintenance activities effectively. These European dimensions are considerably larger than the minimum dimensions stated for the housing of cats in research facilities in the United States, where a 4 kg cat can be housed in an enclosure with 4 square feet (0.37 m^2) of floor space and a height of 2 feet (0.6 m) (National Research Council, 2011). In the author's opinion, the National Research Council minimum dimensions are too small; the minimum floor space requirement for cats should be determined by their socio-spatial needs rather than by their body weight. Research facilities should aim to exceed these dimensions in order to be able to create enclosures that are well designed to meet the cats' needs.

Minimum space requirements for the housing of cats, Appendix A to the European Convention ETS 123 (Council of Europe, 2006)

	Floor area (excluding shelves) m^2	Shelves m^2	Height m
Minimum for 1 adult cat	1.5	0.5	2
For each additional cat add	0.75	0.25	

The minimum space in which a queen and litter may be held is the space for a single cat, which should be gradually increased so that by 4 months of age litters have been re-housed to conform with the above space requirements for adults.

Once sufficient floor space is provided, it is the overall complexity and vertical space availability that are of greatest importance. Cats are agile and athletic animals who enjoy exploring, climbing, running, and jumping—spending more time off than on the floor. Structures that enable cats to make maximal use of the vertical dimension include climbing towers, climbing frames, raised walkways, "cat aerobic centers," and platforms or shelves placed at different heights. Slanting boards and steps will help kittens and small cats to reach the raised areas.

Cats spend long periods of time sleeping, dozing or resting. They prefer soft resting substrates, such as pillows or fleece beds (Crouse et al., 1995; Hawthorne et al., 1995) and materials that maintain a constant temperature (Roy, 1992).

Hiding is a coping behavior that cats frequently display in response to changes in their environment or to avoid interactions with other cats or people. Being potential prey as well as predator, and in order to avoid conflict and reduce stress, they prefer to be partially or completely hidden and, wherever possible, off the floor and preferably in a corner. This allows them to monitor their surroundings without being exposed or attacked from behind. These hiding and resting places, or "safe havens," must therefore provide concealment and height as well as comfort. Hammocks, high-sided trays, "igloos," cardboard or plastic boxes and similar structures, placed securely on raised shelves and ledges as well as on the floor, are all suitable. Various types of plastic children's furniture are often effective, inexpensive and can easily be cleaned. While cats don't particularly favor this substrate, it can be improved by the addition of soft bedding.

There should be more resting and hiding places in the enclosure than there are cats, in order to minimize competition and also because some cats like to change places. Cats will show individual preferences, and these should be identified and met wherever possible. Visual barriers, such as vertical panels, are very useful to divide the enclosure into separate spaces, enabling cats to make choices and to avoid others.

Whenever possible, cats should be housed as pairs or in groups. In some instances, it may be necessary to house a cat singly for recovery after a clinical procedure or for metabolic studies. This should be for as short a time as possible. The cage should still have at least 1.5 square meters of floor space, but it does not have to be walk-in. Ideally, it should be no less than 1 meter high so that the cat can stretch fully in the vertical direction, and a shelf must be installed in such a way that the cat can comfortably rest on it and freely move on and under it. A hiding box should also be provided, placed under or securely on the shelf. If there isn't room for a hiding box, a towel attached to the shelf so that it acts as a curtain for the space below, or a towel covering half of the front of the cage, are ways to create a hiding space for the cat.

Cages should not be stacked one on top of the other, and placing them on a shelf at waist height will make access easier for the caretaker. Methods to join two small cages together via a porthole to make a larger single cage have been described (UC-Davis Koret Shelter Medicine Program, 2010). Cats who have to be housed singly for more than 2–3 days should be allowed daily access to a larger enclosure where they can explore, play, and interact more naturally with the caretaker. Queens in the last 2 weeks of pregnancy, and queens with unweaned kittens should not be housed with other cats.

The floor of all cat enclosures should be smooth, nonslip and easy to clean. Wire-mesh or grid floors are not suitable, as they are uncomfortable for cats and may trap and injure their extremities (toes, paws and tails).

Social

Positive interactions with humans and other cats should continue beyond the sensitive period of social development (typically between 2 and 8 weeks of age) (Karsh & Turner, 1988) throughout the cats' lives. Periods of time each day should be set aside for interaction among cats and their caregivers (Loveridge, 1994; Rochlitz, 2005).

Most cats should be housed in groups providing that there is sufficient space, easy access to feeding and elimination areas, and an adequate number of hiding retreats and resting places. Many factors will determine the ideal group size, but it seems that 20 to 25 individuals is the maximal number for cats in laboratories (Hubrecht & Turner, 1998), although European guidelines recommend groups of up to 12 cats (Council of Europe, 2006). Cats who fail to adapt satisfactorily to living in groups should be identified and housed in compatible pairs or, if incompatible, singly. It is particularly important that singly housed cats receive additional daily human contact and, if judged to be beneficial, visual contact with other cats.

Neutered cats can be kept together in groups, as can intact females. While some authors suggest that intact males should be housed singly, others have shown that they can be housed successfully with other intact and neutered males (Podberscek et al., 1991); they can also be kept with neutered females.

The introduction of a new cat to a group should be done slowly and under supervision (Rochlitz, 2009). Initially, the cat should

be kept in a small cage placed within the group's enclosure. It is vital for the cat to have a box to retreat to, in order to escape the attention of the resident cats. Usually within 2 weeks, the newcomer can safely be released into the enclosure. The use of a synthetic analogue of naturally occurring feline facial pheromones, such as Felifriend (CEVA Animal Health Ltd.), can facilitate the introduction of an unfamiliar cat into an established group (Pageat & Tessier, 1997; Mills, 2005).

Sensory

Marking behaviors: Scratching on surfaces helps to maintain the claws, stretch the body, and exercise muscles and tendons in the legs. It is also a means of visual and olfactory communication. In addition to leaving visual striations or lines, claw scratching deposits scent from sebaceous glands in the cat's paws and is a form of marking behavior. Most cats are strongly motivated to scratch, so scratching posts, disks, rush matting, hessian, wood, or similar surfaces should be provided (Rochlitz, 2009). They should be placed in a number of locations throughout the enclosure, especially near entry/exit points and resting places, and replaced when worn. Some cats prefer to scratch on vertical surfaces, while others prefer horizontal surfaces. Scratching surfaces should be long enough so the cat can stretch fully.

Cats also scent mark by rubbing the sides of their face against protruding vertical structures such as corners and edges of boxes (as well as allorub with conspecifics). The deposition and exchange of scent are thought to be important in maintaining group cohesion and conveying other information. Excessive cleaning of scent-marked areas can disrupt these familiar and reassuring smells, so spot cleaning may be preferable, providing that adequate hygiene is maintained (Rochlitz, 2009). Another way to maintain olfactory continuity in the enclosure is to wash only a portion of the bedding at a time.

Catnip, provided as a dry herb or in toys, may elicit positive behavioral responses (play, sniffing, pawing), which are seen in 50–70% of cats, as sensitivity to catnip is inherited (Ellis, 2007). As mentioned previously, synthetic pheromone products have been developed for use in cats. Felifriend is used to reduce fear and promote positive interactions among cats, and between cats and humans, while Feliway (also from CEVA) is used to reduce anxiety- and stress-related behaviors such as urine spraying (Mills, 2005; Ellis, 2009).

Visual and auditory stimuli: Seeing what is going on outside the cat's immediate enclosure gives the cat a sense of predictability and control and is often a rich source of stimulation, leading to improved welfare. DeLuca and Kranda (1992) found that cats in a colony spent almost all their time sitting on an indoor window ledge and watching activities in the hallway. External

windows bring in natural light, and outdoor activities are also a source of interest and stimulation. Windows with deep ledges or raised, resting platforms will facilitate comfortable viewing, whether it is towards the indoor or outdoor environment. Visual stimulation using television may hold the cat's attention, especially when prey items (e.g., small rodents, birds) and conspecfics (in amicable situations) are presented (Ellis & Wells, 2008).

Cats are very sensitive to noise and easily startled. It is important for kittens to be exposed to a range of normal sounds during their socialization period and beyond. Noises as familiar background sounds, such as low-volume music, may be reassuring (Loveridge, 1994; Newberry, 1995), but loud or unpredictable noises may cause stress. For this reason, cats should not be housed where they can hear dogs barking or other loud noises.

Occupational

An outdoor enclosure or pen will provide an area for exploration, exercise and stimulation. It should be secure and protected from contact with outdoor cats or other animals. Addition of climbing frames, tunnels, and other furniture will encourage activity. There should be more than one passageway (e.g., cat flap) leading to the outdoor enclosure so that the entry and exit point cannot be guarded and monopolized by one cat.

Play: The expression of play behaviors in cats, whether it is by playing alone or with humans, is usually interpreted as evidence of good welfare (table page 156). Uninterrupted contact periods for caretakers and their cats should be scheduled into the institution's daily routine. Individual cats often have specific likes and dislikes. For example, some enjoy being stroked on the head or being groomed, some prefer noncontact interaction such as playing with humans via a toy, and some dislike being picked up (Soennichsen & Chamove, 2002; Rochlitz, 2005; Ellis et al., 2013). Toys that are small and mobile and have complex surface textures (e.g., fur, feathers) often elicit play (Hall & Bradshaw, 1998), as do simple objects like large paper bags or cardboard boxes (e.g., for "hide and seek" games). Toys should be regularly replaced to maintain interest and ensure their novelty effect (De Monte & Le Pape, 1997; Hall et al., 2001). There is a huge range of items available for playing with cats and some are more effective than others; the caretakers should be able to identify what their cats prefer. Because most cats play alone rather than in groups, the enclosure must be of sufficient size to ensure that cats can play safely without disturbing others.

Nutritional

Feeding: Cats prefer frequent small meals throughout the day and night (this would occur in the wild, as they catch prey sporadically) but are usually offered two meals a day. Ways of increasing the time the cat spends in feeding behaviors—in particular, in performing parts of the predatory sequences prior to consuming food—have been devised. For example, dry cat food ("kibble") can be hidden in the environment for the cat to find. Food can be put in puzzle feeders (or food balls), which are food-dispensing containers for the cat to manipulate. Flat activity boards, where the cat has to manipulate dry food out of obstacles on the device before it can be consumed (mimicking some predatory behaviors), are also commercially available. Because cats do not usually eat together, food bowls should be dispersed throughout the enclosure and placed on raised surfaces as well as on the floor. There should be a sufficient number of bowls so as to avoid conflict or guarding (monopolization). Consideration should be given to the provision of containers of grass for cats to chew, as this is thought to help eliminate fur balls (trichobezoars).

Drinking: Water should be available 24 hours a day, and changed daily to ensure freshness. Factors to consider include movement (e.g., water fountains), shape of container (some cats do not like their whiskers to touch the sides of the container when drinking), and position. Because cats often prefer to drink away from the feeding area and at times unconnected with feeding, there should be bowls of water in several locations, both near and away from food bowls (and outdoors as well as indoors, if cats have outside access).

Toileting (elimination): Cats are fastidious when it comes to toileting behavior. They urinate and defecate in locations that are safe, clean, not near resting and hiding places or areas of activity, and that are not under the constant scrutiny of other cats. Failure to use the litter tray should alert the caretaker to possible health or behavior problems in the group, or to inadequate provision of suitable toilet areas.

Litter boxes should be dispersed throughout the enclosure but, preferably, at least 0.5 to 1 meter away from feeding, drinking and resting/hiding areas, and easily accessible to kittens as well as adults. There should be at least one box per two cats and it should be cleaned at least once, and preferably twice, a day. More frequent cleaning will reduce the number of litter trays required, thereby freeing up available floor space. Ideally the litter box should be longer than 1.5 times the average body length of a cat, so that the cat can reach forward to rake clean litter back over their waste. Often, litter boxes are too short, so large plant trays may be a better choice. The space occupied by the litter box should not be considered as part of the overall floor space.

With regard to type of litter box (covered or not) and type of litter, cats have individual preferences and these should be met wherever possible. Within the litter box it is important that there is sufficient substrate to bury feces, and unscented, fine-grained clumping litter is often preferred. Fine-grained clumping litter should be completely removed weekly because scents and small particles of feces can coat the surfaces of individual grains (Overall & Dyer, 2005).

Refining husbandry and research procedures for cats

Handling: Socialization from a young age, daily positive interactions with familiar and unfamiliar people, and exposure to a complex environment promotes the development of kittens into adult cats that are easier to handle for research procedures. (Hoskins, 1995). Handlers should be empathetic, calm, gentle, and focused; speak in a quiet voice; and approach from the side rather than the front. They should avoid direct or unblinking eye contact with the cat. If the cat is frightened and uncooperative, covering the animal's head or wrapping the body in a towel will help to calm the cat down and make handling safer.

Scruffing (grasping folds of the cat's skin in the cervical area) is a behavior usually seen in cats in only limited circumstances, such as mothers carrying kittens and during mating (and, less commonly, fighting). It is not naturally used as an effective method of discipline (Rodan et al., 2011). Pinching the cat's skin along the back of the neck ("clipnosis") may also inhibit reactions, but it is unclear if this is due to pain or other effects (Tarttelin, 1991; Pozza et al., 2008). Neck scruffing or cervical skin pinching as methods of restraint by humans, therefore, should be avoided whenever possible, until more is known about how the cat experiences these methods. It is important to realize that when cats react negatively to handling it is usually due to fear, so handling methods should aim to reduce fear and gain the cat's trust rather than to escalate to aggression. Guidelines on the humane handling of cats can be found in Rodan et al. (2011).

Training: Most cats can be trained to interact in particular ways in order to make procedures safer and less aversive. Operant conditioning techniques are effective (Lockhart et al., 2013), providing there is sufficient time and the trainer is knowledgeable and patient, and only uses techniques based on reward. Most cats can be trained to tolerate routine procedures such as venipuncture, saliva collection (shown in photo above), nail trimming, tooth brushing, and having their temperature taken (Overall & Dyer, 2005). Fearful cats who react poorly to research procedures or to training should be removed from the program and put up for adoption.

Declawing: Declawing (onychectomy) is a surgical procedure performed in some countries (e.g., the United States) but regarded as a mutilation, and therefore banned, in others (e.g., the United Kingdom). It is likely to cause short-term pain and possibly also longer-term pain, and it may frustrate the cat's motivation to scratch. Cats can be handled safely and take part in a research program without being declawed, and declawing has not been shown to "improve" behavior or the chances for a successful adoption (DiGangi & Levy, 2006), so this procedure should not be performed.

Recognizing stress: Cats need predictability, familiarity and routine. When these are absent, cats' coping abilities are reduced, making them less resilient and more susceptible to stress (Herron & Buffington, 2010). This is highlighted in recent studies on sickness behaviors (Stella et al., 2011, Stella et al., 2014). Sickness behaviors refer to a group of nonspecific clinical and behavioral signs that normally occur in response to infection. These behaviors were noted in healthy colony-housed cats in response to environmental disturbances such as transient discontinuation of contact or interactions with the cats' primary caretaker, changes in time of day of routine husbandry, unfamiliar caretakers, and a delay in feeding time. The most common sickness behaviors observed were vomiting of hair, food, or bile; decreased appetite; and eliminating out of the litter tray. While some cats experiencing stress will show overt signs such as sickness behaviors, others will react differently, responding

Some behavioral measures of good and poor welfare in domestic cats in research institutions (modified from Rochlitz 2009, 2014)

Behavior	Good welfare	Poor welfare
Maintenance behaviors[1]	Normal levels	Reduced levels or absent; sickness behaviors
Activity, exploration and investigation of surroundings	Normal levels	Reduced levels or absent (rarely, high levels)
Social interactions with other cats in the group	Present; positive (affiliative) behaviors such as allorubbing, allogrooming, staying in proximity	Absent or negative; hostility, aggression, avoidance of each other
Interactions with caretakers	Initiates positive interactions with caretakers; positive response to initiation of interactions by caretakers	Failure to initiate interactions with caretakers; absence or negative response to initiation of interactions by caretakers
Types of behaviors shown	Shows a wide range of normal behavioral repertoire; friendly behaviors (e.g., tail-up position, rubbing, vocalization)	Persistent signs of timidity, anxiety, fear or aggression; hiding or attempting to hide for long periods; over-grooming; self-mutilation; excessive vocalization; excessive vigilance; feigned sleep[2]
Play	Presence of play (on own, with objects, with other cats, or with caretakers)	Absence of play

[1]Maintenance behaviors: feeding, drinking, grooming, claw scratching, resting, sleeping, urination, defecation.
[2]Feigned sleep: the cat appears to be asleep or resting (body is in sleep posture and eyes are closed or partly closed) but is awake and vigilant.

to poor environmental conditions and disturbances by becoming inactive and reducing normal behaviors such as self-maintenance (feeding, drinking, grooming, claw scratching, resting, sleeping, urination, defecation), exploration, or play (McCune, 1992; Rochlitz, 1999; Casey & Bradshaw, 2007). Some behavioral measures of good and bad welfare in cats are summarized in the table on the previous page.

Source of cats

Cats should be obtained only from designated breeding establishments (class A dealers). Because the sensitive period of socialization is so early in cats (2 to 8 weeks of age), an informed and well-planned socialization program should be in place at the breeder. During this period it is particularly important that the kitten has social contacts with other kittens (e.g., litter mates) and with humans, and is exposed and habituated to the environmental conditions the kitten will subsequently encounter. In addition, the breeding program should take into account that friendliness (also described as boldness) to humans is, in part, genetically inherited from the father. Kittens from friendly fathers tend to react with greater boldness when faced with unfamiliar people and novel objects (McCune, 1995).

Final thoughts

Cats respond strongly to humans in their environment and organize their daily activity patterns around the caretakers' activity, preferring human contact over toys (Randall et al., 1990; DeLuca & Kranda, 1992). Caretakers should like cats, and be knowledgeable of their behavior and reactions to stress so that signs of poor welfare, whether overt or more subtle, are recognized promptly. However, it should be recognized that even though the caretakers are key components to a cat's welfare, they are not a substitute for a proper physical environment. High standards of housing, enrichment, management, and human interaction will create an optimal scenario for the cat in research.

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Nonhuman Primates

Viktor Reinhardt, DVM, PhD

THE DESIGN OF species-adequate housing conditions and humane handling practices for nonhuman primates in research laboratories must take the following facts into account:

1. *In their natural habitat,* nonhuman primates maintain vocal, visual and/or tactile contact with other conspecifics; spend most of the day foraging, i.e., searching for, retrieving and processing food; show a vertical flight response during alarming situations, and retreat to high places during the night (Napier & Napier, 1994).
2. *In the research laboratory,* nonhuman primates experience anxiety (distress) before and intense fear (stress) during enforced restraint procedures (Reinhardt et al., 1995; Reinhardt, 1998).

Animal Welfare Concerns of Traditional Housing and Handling Practices

Alone in a boring enclosure: The inadequacy of housing a nonhuman primate alone in a boring enclosure is addressed by the following professional guidelines and legislative rules:

The International Primatological Society (1993 & 2007)

1. Pair or group housing in an enclosure must be considered the norm. For experimental animals, where housing in groups is not possible, keeping them in compatible pairs is a viable alternative social arrangement. A compatible conspecific probably provides more appropriate stimulation to a captive primate than any other potential environmental enrichment factor. Single caging should only be allowed where there is an approved protocol justification on *veterinary or welfare grounds* [emphasis added].
2. As monkeys and apes like to work for their food (Köhler, 1921; Yerkes & Yerkes, 1929; Murphy, 1976; Anderson & Chamove, 1984; Evans et al., 1989; Line et al., 1989; Menzel, 1991; Washburn & Rumbaugh, 1992; O'Connor and Reinhardt, 1994; Reinhardt, 1994a; Inglis et al., 1997; Taylor, 2002; de Rosa et al., 2003), increasing processing time, increasing foraging, or providing puzzle feeders or other feeding devices is encouraged.
3. The vertical dimension of the cage is of importance [because of the vertical flight response] and cages where the monkey is able to perch above human eye level are recommended.

The Primate Research Institute (2003) of Japan

1. Primates are very social animals. Physical contact, such as grooming, and noncontact communication through visual, auditory, and olfactory signals are vital elements of their lives. Providing animals with a satisfactory social interaction helps to buffer against the effects of stress, reduce behavioral abnormalities, increase opportunities for exercise and helps to develop physical and social competence.
2. Food presentation should satisfy the animal's interest in manipulating objects. In order to satisfy their requirement to interact with their environment, it is desirable to provide feeders that require complex handling or devices which in some way lead the animals to object manipulation.
3. Devices suitable for gross motor and behavioral patterns, such as perches and three-dimensional structures should be arranged to make as much use of the available space as is possible. Diversity is essential to the housing environment of laboratory animals. Windows through which the animals can see the outside world may help to alleviate some boredom.

The Medical Research Council (2004) of the United Kingdom

1. Primates should be socially housed as compatible pairs or groups. They should not be singly housed unless there is *exceptional* [emphasis added] scientific or veterinary justification.
2. The MRC will require *justification for the use of scientific procedures that restrict the opportunity to forage* [emphasis added].
3. The volume and height of the cage (or enclosure) are particularly important for macaques and marmosets, which flee upwards when alarmed. Their cages and enclosures should be floor-to-ceiling high whenever possible, allowing the animals to move up to heights where they feel secure. Cages and enclosures should be furnished to encourage primates to express their full range of behaviours. Depending on the species, this should normally include provision for resting, running, climbing and leaping.
4. Primates *must* [emphasis added] be provided with a complex and stimulating environment that promotes good health and psychological well-being and provides full opportunity for social interactions, exercise and to express a range of behaviours appropriate to the species.

The Canadian Council on Animal Care (1984 & 1993)

Any primate housed alone will probably suffer from social deprivation, the stress from which may distort processes, both physiological and behavioural. In the interest of well-being, a social environment is desired for each animal which will allow basic social contacts and positive social relationships. Social behaviour assists animals to cope with circumstances of confinement.

The United States Department of Agriculture (1991)

Dealers, exhibitors, and research facilities must develop, document, and follow an appropriate plan for environmental enhancement adequate to promote the psychological well-being of nonhuman primates. The plan *must* [emphasis added] include specific provisions to address the social needs of nonhuman primates of species known to exist in social groups in nature. The physical environment in the primary enclosures *must* [emphasis added] be enriched by providing means of expressing noninjurious species-typical activities. Examples of environmental enrichment include providing perches, swings, mirrors, and other increased cage complexities; providing objects to manipulate; varied food items; using foraging or task-oriented feeding methods; and providing interaction with the care giver or other familiar and knowledgeable person consistent with personnel safety precautions.

The National Health and Medical Research Council (1997) of Australia

For nonhuman primates social interaction is paramount for well-being. Social deprivation in all its forms *must* [emphasis added] be avoided. Animals that need to be individually caged, either for experimental or holding purpose (for example, aggressive adult males), must be given contact with conspecific animals. Accommodation should provide an environment which is as varied as possible. It should meet the behavioural requirements of the species being used. Emphasis *must* [emphasis added] be placed on environmental enrichment.

The Council of Europe (2006)

Because the common laboratory nonhuman primates are social animals, they should be housed with one or more compatible conspecifics. Single housing should only occur if there is justification on *veterinary or welfare grounds* [emphasis added]. The structural division of space in primate enclosures is of paramount importance. It is essential that the animals should be able to utilise as much of the volume as possible because, being arboreal, they occupy a three-dimensional space. To make this possible, perches and climbing structures should be provided.

Enforced restraint: Nonhuman primates experience intense fear when they are forcibly subjected to handling procedures. It may be true that procedures such as injection and blood sampling are simple, but they can be expected to "produce little or no discomfort" (Scientists Center for Animal Welfare, 1987) *only* if the subject is *not* forced to leave her or his cage and subsequently is *not* forced to hold still during such a procedure.

The problems associated with involuntary restraint are addressed by the following professional guidelines and legislative rules:

The International Primatological Society (2007)
Primates of many species can be quickly trained, using positive reinforcement techniques, to cooperate with a wide range of scientific, veterinary and husbandry procedures. Such training is advocated whenever possible as a less stressful alternative to traditional methods using physical restraint. Techniques that reduce or eliminate adverse effects not only benefit animal welfare but can also enhance the quality of scientific research, since suffering in animals can result in physiological changes which are, at least, likely to increase variability in experimental data and, at worst, may even invalidate the research. Restraint procedures should be used only when less stressful alternatives are not feasible.

The Primate Research Institute (2003) of Japan
Pain and other physical stress, such as physical or chair restraint, most definitely affect the behavior and psychology of laboratory animals. All possible measures to reduce their incidence should be taken. Animals should be trained to be as cooperative as possible to the procedures to facilitate the rapid completion of work and to alleviate stress in both the animals and people in charge.

The Home Office (1989) of the United Kingdom
The least distressing method of handling is to train the animal to co-operate in routine procedures. Advantage should be taken of the animal's ability to learn.

Enforced restraint is sometimes advocated with the assertion that nonhuman primates are unpredictable and readily scratch and bite handling personnel (Gisler et al., 1960; Ackerley & Stones, 1969; Valerio et al., 1969; Altman, 1970; Whitney et al., 1973; Henrickson, 1976; Wickings & Nieschlag, 1980; Robbins et al., 1986; Wolfensohn & Lloyd, 1994; Johns Hopkins University and Health System, 2001; Panneton et al., 2001; University of Arizona - IACUC Certification Coordinator, 2008; University of Minnesota - Investigators, and Animal Husbandry and Veterinary Staff, 2008). This contention overlooks the fact that the animals are not intrinsically "aggressive," but that enforced restraint *makes* them aggressive. Trying to bite or scratch the handling personnel is the biologically normal self-defense of any animal who is forcibly restrained. The very act of forceful restraint triggers, rather than prevents, aggressive self-defense. Gaining the animal's trust, and then training him or her to cooperate—instead of resist—during procedures eliminates the risks that are associated with self-defensive aggression. A cooperative animal is no longer given any reason to bite or scratch the investigator, animal technician, animal caretaker or veterinarian who is working *with*, rather than against, the animal during a procedure.

Refinement » Social enrichment

Animate enrichment addresses the social needs of nonhuman primates by promoting noninjurious contact and interaction with one (pair-housing) or several (group-housing) compatible conspecifics.

Establishing groups: Given that nonhuman primates are social animals, it is vital to address their need for compatible companionship when they are kept in research laboratories (United States Department of Agriculture, 1991). Housing the animals in compatible groups is the most species-appropriate strategy to address their social needs.

There are numerous reports on integrating animals into already established groups, but only a few on forming a new group of previously single-caged individuals. Fritz & Fritz (1979) and Fritz (1994) developed a protocol to introduce previously single-caged **chimpanzees** to unfamiliar peers. The newcomer is first moved into a specially designed social unit and kept next to the cage of a selected member of an already established group. The two chimpanzees have full olfactory, visual and auditory contact, as well as limited tactile contact. The selected group member is moved in as a cage mate for the newcomer as soon as friendly interactions through the separating cage mesh are consistently observed. After several days, another group member is introduced to the pair in this same way, then another is introduced to the trio, and so on until the newcomer has met all members of the group and is then fully integrated. A total of 59 of 60 chimpanzees of both sexes and all age classes were successfully socialized to compatible group-living in this manner, without a single incidence of serious fighting.

Kessel & Brent (2001) tranquilized adult single-caged **baboons** with ketamine and placed one trio of males in one enclosure and two trios of two females and one male in two other enclosures, where the animals regained consciousness. The formation of the three groups was accompanied by two incidences of wounding, which were superficial and required no medical treatment. Bourgeois & Brent (2005) confirmed these findings in a subsequent study with three adolescent male baboons. Group formation was accompanied by no overt aggression. Rough-and-tumble wrestling was observed and dominance was quickly established.

Bernstein & Mason (1963) released 11 **rhesus macaques** (three adult females, two adult males, one subadult female, one subadult male and four juveniles) simultaneously into a large enclosure. During the first hour, a total of 83 threats and 23 attacks were observed; injurious encounters were not recorded, but one of the two males soon showed signs of deteriorating health and died after 20 days. Gust et al. (1991) simultaneously introduced eight unfamiliar adult female rhesus macaques and one unfamiliar adult male in a large enclosure. There was no serious fighting, and in fact no contact aggression was recorded, even though firm dominance-subordinance relationships were established during the first 48 hours. Several females stayed in close proximity of the male, who copulated with two of them during the first day. The male's presence probably accounted for the females' tolerance of each other (Bernstein, 2007). Reinhardt (1991a) tried to form an isosexual rhesus macaque group consisting of six previously single-caged adult females and another group of six previously single-caged adult males. Future group members were first given ample opportunity to physically interact with each other on a one-to-one basis during a 1-week period. Dominance-subordinance establishment was ascertained in each dyad. The two groups were then formed by releasing the six animals simultaneously into a big cage. In both situations, aggressive incompatibility was heralded by certain subjects challenging other partners to whom they had been subordinate during the familiarization week. Aggressive harassment was intense and persistent. Alliances were quickly formed and several animals in union attacked selected targets. Victims were cornered, and they showed no resistance, except for fear-grinning and submissive crouching; they did so to no avail and the vicious attacks continued. Both groups were disbanded within the first hour to avoid fatal consequences.

Gust et al. (1996) introduced eight adult female and one adult male **pig-tailed macaques** simultaneously in a large enclosure: Group formation and the establishment of a social hierarchy were not associated with serious aggression; there was no contact aggression during the first 5 hours following the simultaneous release of the eight animals into the same enclosure. The presence of the male probably functioned as an aggression buffer between the females (Dazey et al., 1977).

Clarke et al. (1995) familiarized three single-caged adult male **long-tailed (cynomolgus) macaques** pairwise with each other in a noncontact housing arrangement for 2 weeks and subsequently released them as a trio in a large cage. No injurious fighting was recorded; the new group was compatible. Asvestas (1998) and Asvestas & Reininger (1999) established a group of 22 adult male long-tailed macaques by first forming 11 compatible pairs. After 9 months, all animals were sedated with ketamine and placed simultaneously in a big enclosure where they regained consciousness under careful supervision of the attending staff. The new group turned out to be compatible, even though four males were slightly injured during fighting.

Clarke et al. (1995) kept three male **lion-tailed macaques** in a housing arrangement that allowed all animals to see each other for a period of 2 weeks. The males were subsequently released simultaneously into a large cage. This event was not accompanied by serious fighting, but the group was disbanded because the three males avoided each other and were apparently sufficiently distressed that their well-being was compromised, especially that of the lowest-ranking animal, who did not obtain sufficient food. Stahl et al. (2001) released six unfamiliar adult lion-tailed macaques into well-structured, large living quarters and encountered no aggression-relation problems. The six males showed 91 noncontact agonistic interactions but no physical aggression during the first 6 hours.

King & Norwood (1989) released 11 single-caged female and 13 single-caged male **squirrel monkeys**, ranging in age from 1 to 18 years, without any preliminaries, into a well-structured room. The establishment of the new group was accompanied by two deaths—one male and one female—resulting from attacks by other monkeys.

No foolproof recipe is yet available for group formation of **capuchin monkeys**. Our knowledge of how to form capuchin groups does not come from systematic experimental study, but derives from husbandry problems faced occasionally by laboratories. Overall, group formation is a stressful procedure both for the animals and the caregivers, and although cumulative experience may help to reduce the risks of failure, the outcome can never be predicted with certainty (Visalberghi & Anderson, 1999).

Group-housing: Housing three or more nonhuman primates together in the same enclosure can bear substantial risks for individual members of the group, especially when mature animals of both sexes are present. The inherent constraints of confinement often make it impossible for individuals to keep appropriate social distance from each other, so as to avoid conflicts. Research-related and management-related interferences in the group's membership are bound to destabilize its social structure, thereby triggering rearrangements in the social hierarchy that are usually associated with overt aggression and social distress (Southwick, 1967; Kaplan et al., 1980; Kessler et al., 1985; Cohen et al., 1992; Visalberghi & Anderson, 1993; Alford et al., 1995; de Filippis et al., 2009).

Serious, sometimes fatal injuries resulting from aggression are not uncommon in captive groups of baboons (Rowell, 1967; Nagel & Kummer, 1974), chimpanzees (Alford et al., 1995), squirrel monkeys (Abee, 1985), marmosets (Poole, 1990), vervet monkeys (Knezevich & Fairbanks, 2004), pig-tailed macaques (Sackett et al., 1975; Erwin, 1977), and rhesus macaques (Kaplan et al., 1980; Kessler et al., 1985; Schapiro et al., 1994), Rolland (1991) notes that "By far the most common physical problem that I treat as clinical veterinarian is trauma sustained by macaques in group-housing situations."

No published report could be found of serious aggression problems in core groups of long-tailed macaques (cf., Aureli et al., 1993; Clarke et al., 1995; Ljungberg et al., 1997), stump-tailed macaques, mangabeys, capuchin monkeys (cf., Fragaszy et al., 1994), and tamarins (cf., Poole et al., 1999).

Successfully transferring single-caged primates to compatible group-housing can be an effective remedy for self-injurious biting. Fritz (1989) observed that an unspecified number of chimpanzees gradually stopped biting themselves after they were transferred from single- to compatible group-housing arrangements. Alexander & Fontenot (2003) noted that 10 adult male rhesus macaques, who engaged in self-injurious biting while they were single-caged, showed no signs of this behavioral pathology during the first four months after they were transferred to compatible isosexual group-housing.

Establishing pairs: "To enhance the life-style of a primate, one of the most effective, but often overlooked improvements is pair-housing" (Rosenberg & Kesel, 1994, p 469). Keeping nonhuman primates in compatible pairs is a good compromise to group-housing; it addresses the animals' basic social needs while providing more assurance of their safety, better access to individuals, and control over their reproduction. Initial, strong reservations against the transfer of single-caged animals to pair-housing arrangements have proven to be based on the erroneous idea of the aggressive and near-intractable monkey (Gisler et al., 1960; Bernstein et al., 1974; Wickings & Nieschlag, 1980; Line, 1987; Coe, 1991; Rosenberg & Kesel, 1994) and the disregard of basic ethological principles when establishing new pairs.

Adults with juveniles: Adults—both females and males—are normally inhibited from showing overt aggression toward juveniles. This circumstance makes it unproblematic to transition single-caged adults to compatible pair-housing arrangements: the naturally weaned juvenile is simply introduced with the adult in the adult's home cage. Typically, the adult will show parental responses, huddling with the young, spending much time grooming the young, and allowing the young to engage in often exuberant play behaviors. Even rhesus males, who have the reputation of being particularly aggressive (Wickings & Nieschlag, 1980), have the tendency to treat their little companions with gentleness and great tolerance. Reinhardt (1994b) transferred naturally weaned, surplus juveniles between the ages of 12 and 18 months from a rhesus macaque breeding colony without any preliminary precautions, pairwise to unfamiliar single-caged adults of both sexes. A total of 78 pairs were tested and pair compatibility ascertained during the first week in 96% (75/78) of cases: the adult did not injure the juvenile, the juvenile showed no signs of depression, and the adult shared food with the juvenile. Three pairs (4%) were incompatible. One female grabbed her female juvenile immediately upon her arrival; she continued to do this repeatedly during the next 30 minutes, after which the youth was removed. One male bit his male juvenile on the fourth day of introduction. The youngster was slightly injured, although not bleeding. When the juvenile started to consistently avoid the adult, the pair was split. Another male often grabbed his little male companion, even though he gently groomed him, and the two huddled with each other regularly. Gradually, however, the juvenile showed more and more avoidance behavior, and the two were finally separated after 9 days.

Juveniles with juveniles: Juveniles who have not yet reached the age when they become ambitious to dominate over others are usually compatible when they are introduced as pairs, even when they are strangers to each other. Reinhardt (1994b) transferred a total of 84 female and 22 male juvenile rhesus macaques to same-sex pair arrangements. All pairs were compatible throughout a 1-year follow-up period. Males were occasionally observed playfully wrestling with each other, but this never resulted in injurious aggression or depression.

Adults with adults: Adult primates have the tendency to react with hostility when they meet another adult conspecific with whom they are not familiar. Strangers first determine their dominance-subordinance relationship; this often involves potentially injurious fighting. To avoid this in the laboratory setting, single-caged adults assigned to be paired with another adult partner are usually first given the opportunity to get to know each other during a noncontact familiarization period.

Reinhardt et al. (1988) and Eaton et al. (1994) familiarized previously single-caged adult, female **rhesus macaques** in double cages with transparent partitions for 1 week, and then introduced them as pairs in a different double cage. Within the first 2 hours after introduction, dominance-determining fighting was witnessed in 27% (5/18) and 10% (2/21) of cases, respectively. The fights resulted in no serious injuries, but they were persistent and led to depression in the victim, in three dyads of the 1988 study and in two dyads of the 1994 study. These five pairs were classified as incompatible and the partners were permanently separated; consequently, pair compatibility during the first week was 83% (15/18) and 90% (19/21), respectively.

Reinhardt (1994b) made sure that the partners of 77 adult female rhesus dyads and 20 adult male rhesus dyads had established their dominance-subordination relationships during a noncontact familiarization period, before they were introduced in a different double cage.

This precaution was implemented in order to minimize the animals' need to engage in dominance-determining aggression upon being introduced to each other. The following gestures, reactions and behaviors were taken as indicators that one animal was subordinate and accepted the dominant position of his or her neighboring partner:

a. strictly unidirectional fear-grinning when being looked at by the neighbor,
b. withdrawing and/or looking away when being approached or looked at by the neighbor, and
c. quickly glancing at the neighbor followed by threatening against other animals of the room or against the observer.

Partners who had established such a relationship were then introduced to each other in a different double cage to avoid potential territorial antagonism (Reinhardt et al., 1988; Niemeyer et al., 1998). Newly formed pairs were regularly observed during the first week. Shortly after introduction, fighting took place in only 2 of the 97 dyads tested. Partners turned out to be compatible in 95% (73/77) of the female pairs and also in 95% (19/20) of the male pairs. Compatible partners did not engage in serious aggression, they shared both standard and supplemental food, and none of them became depressed. Within the first week, 5 of the 97 pairs turned out to be incompatible because of injurious aggression (two female pairs), depression (one male pair), or food monopolization (one female pair and one male pair). Doyle et al. (2008) demonstrated in eight biotelemetry-instrumented adult male rhesus macaques who were carefully familiarized in pairs that compatible partners showed no increased heart rate when they were introduced, suggesting that the pair-formation process was not a stressful experience for them.

Lynch (1998) tested 17 adult male **long-tailed macaque** dyads. Potential pairs had all established clear-cut dominance-subordinance relationships during a noncontact familiarization period; subsequent partner introduction was accompanied by fighting in only one incompatible pair. The other 16 pairs (94%) were compatible. Crockett et al. (1994) also familiarized the potential partners of 15 adult male and 15 adult female long-tailed macaques in a noncontact housing arrangement, but introduced the animals as pairs in the familiarization cage without prior verification that they had established dominance-subordination relationships. Under these circumstances, fighting occurred shortly after introduction in 67% (10/15) of the male pairs and in 13% (2/15) of the female pairs.

Reinhardt (1994c) transferred 10 adult female and six adult male **stump-tailed macaques** from single-housing to isosexual pair-housing by first allowing potential partners to establish dominance-subordinance relationships without risk of injury, during a noncontact familiarization phase. Following subsequent introduction in a new home cage, all eight pairs showed signs of compatibility. Female partners reconfirmed their rank relationships within 30 minutes with subtle gestures, never by overt aggression. Male partners engaged in hold-bottom rituals, whereby one puts both hands on the other's hips (de Waal & Ren, 1988) upon being introduced to each other. Two male pairs reconfirmed rank relationships within 30 minutes with gestures, while the third pair resorted to a brief noninjurious dominance-reconfirming fight, which was followed by another reconciliatory hold-bottom ritual.

Coe & Rosenblum (1984) introduced 10 unfamiliar, adult male **bonnet macaques** pairwise without any preliminaries. As usually occurs when unfamiliar males first meet, agonistic behaviors related to the establishment of dominance relations occurred at pair formation. The aggressive incidents were limited, usually involving threats and pursuit behavior; manual attacks occurred only infrequently. More typically, one animal submitted and indicated his subordinate status through communicative gestures. In the first week following pair formation, the occurrence of aggressive behavior subsided almost entirely. The males' response to this pairing procedure may reflect their reputation for showing the highest degree of male-male tolerance in the genus *Macaca*.

Bourgeois & Brent (2005) established four pairs of previously single-caged subadult male **baboons** by sedating potential companions and having them wake up together in the same cage. No serious aggression was witnessed during ten 30-minute observations conducted during the first 2 weeks. Jerome & Szostak (1987) allowed an unspecified number of adult female baboons to live pairwise with each other 4 hours a day, three times a week. The same pairs visited each other in either animal's cage. No overt aggression occurred during visits.

Majolo et al. (2003) checked the clinical records of 56 unfamiliar female **common marmosets** of different age classes who were paired with each other without familiarization. Overall, 22 (79%) out of 28 pairs were compatible; the other six pairs were split up within the first week after pair formation because one of the monkeys was subjected to intense aggression and/or was injured as a consequence of fighting.

Pair-housing: Reinhardt (1994b) formed 84 compatible pairs of juvenile female and 22 compatible pairs of juvenile male **rhesus macaques** and noted that the animals remained compatible for at least 12 months. There were 21 juvenile female pairs with cranial implants. Living together in the same cage did not constitute any specific risks for the animals (no local infections possibly caused by grooming the margins of the implantation site; cf., Anonymous, 2007) and no risk for the implants (no damage related to social interactions; cf., Anonymous, 2007).

Reinhardt (1994b) created 75 compatible adult-infant pairs who were allowed to stay together uninterruptedly. Compatibility was ascertained throughout a 12-month follow-up period. Incompatibility was noted after more than 1 year in two cases, when the now prepubertal young subjects started teasing their over-30-year-old companions, thereby creating excessive disturbance for these aged animals. Two of the infants lived with adult females who were tethered, and 32 paired infants had cranial implants. Both circumstances did not interfere with research protocols requiring remote sample collection and neuroendocrinological testing (cf., Reinhardt & Dodsworth, 1989).

Doyle et al. (2008) allowed eight, previously single-caged adult male rhesus macaques to live uninterruptedly as four compatible pairs. Over the course of 18 months, one bite laceration was incurred (after 3.5 months), but the pair remained compatible after the injury was treated and healed. Average fecal cortisol levels were significantly higher when the males lived alone than after they had lived with a companion for 20-39 weeks (83 ng/g versus 9 ng/g), indicating that long-term pair-housing was a less distressing situation than single-housing. Scan sampling revealed that the males groomed each other about 13% of the time.

Eaton et al. (1994) studied 12 newly formed, compatible adult female rhesus pairs over a 36-month period. During this time, only one pair became incompatible, when the two partners had a serious fight. Compatible companions groomed each other during about 30% of multiple 10-minute observation sessions. Reinhardt (1999) worked with three adult female and four adult male rhesus macaques who habitually bit themselves when they were caged alone. The provision of perches, gnawing sticks, and food puzzles did not alleviate this behavioral pathology, but when the seven animals were successfully paired with compatible partners, the self-biting stopped immediately in three cases and gradually in the remaining four cases. Baker et al. (2012) noted in 46 adult female and in 18 adult male rhesus macaques a statistically significant decrease in abnormal behavior (females: 54%; males: 18%) and anxiety-related behavior (females: 35%; males: 41%) 4 weeks after the animals had been transferred from single-housing to compatible pair-housing condition.

Reinhardt (1990b) assessed the clinical records of a rhesus macaque colony consisting of 237 single-housed and 382 pair-housed animals of both sexes and all age classes. The incidence of non-research-related veterinary treatment was 23% for single-caged animals, versus 10% for pair-housed animals, indicating that the animals' physical health was not jeopardized by sharing a cage with a companion. Schapiro & Bushong (1994) examined the clinical records of 98 juvenile rhesus macaques during 1 year when they were caged alone and the subsequent year when they lived in opposite-sex pairs. Individuals required veterinary treatment more than twice as often when they were single-housed (0.40 times/year) than when they were pair-housed (0.17 times/year). These findings were confirmed in a subsequent study with adult rhesus macaques in which pair-housed animals required significantly fewer medical interventions for diarrhea than did single- or group-housed animals (Schapiro et al., 1997).

Reinhardt (1990a & 1994b) formed 73 compatible adult female and 19 compatible adult male rhesus macaque pairs. The animals were allowed to live together uninterruptedly.

- Over a 12-month follow-up period, compatibility was 93% (68/73) for the female pairs and 84% (16/19) for the male pairs.
- During 60-minute video recordings of eight female pairs and four male pairs, females groomed each other 25% and hugged each other 4% of the time while males groomed each other 12% and hugged each other 2% of the time; the sex differences were statistically significant.
- Among the compatible pairs were four 30- to 35-year-old animals who were so old that they experienced a progressive loss in body weight. Living with a companion did not accelerate this biological process (Vertein & Reinhardt, 1993), suggesting that the permanent presence of a companion did not jeopardize their general health. These elderly rhesus macaques groomed each other, on average, during 22% of multiple 1-hour observations (Reinhardt & Hurwitz, 1993).
- Some animals were assigned to controlled food intake studies over the course of the first 2 years after pair formation. When this happened, they were allowed to stay in their home cage, where they were separated from their companions with a grated cage dividing panel during the day, and reunited for the night after food intake was recorded.
- The majority of the animals were assigned to a timed breeding program. All 18 females who gave birth during the first 2 years after pair formation were allowed to stay with their partners. The presence of offspring did not affect the compatibility between the two cage companions.
- There were 23 female pairs with one or both partners having cranial implants. This circumstance did not jeopardize the integrity of ongoing neurophysiological research of

one or both animals (cf., Truelove, 2009). Evidence shows that pair-housing provides a safe and practical social alternative to single-housing not only for juvenile and adult female macaques but also for adult male macaques with biomedical implants (Roberts & Platt 2004).

» When one partner had to be chair-restrained during an experiment, the companion was brought along in a mobile cage to provide emotional support (cf., Reinhardt & Dodsworth, 1989).

Crockett et al. (1994) established 15 compatible adult female and 12 compatible adult male **long-tailed macaque** pairs and housed them in such a way that partners were separated each day for 17 hours and subsequently reunited for 7 hours. While 100% of the female pairs successfully coped with this situation and remained compatible, only 50% of the male pairs adjusted; the other 50% became incompatible and had to be separated within 2 weeks of living together under these conditions. Lynch (1998) also formed 16 compatible adult male long-tailed macaque pairs, but partners could stay together without interruption. All pairs remained compatible throughout a 12-month follow-up period and longer.

Line et al. (1990) transferred five adult female long-tailed macaques to compatible pair-housing arrangements and observed each pair during daily 10-minute sessions throughout the first two weeks after pair formation. During these sessions companions groomed each other, on average, 31% of the time. The incidence of abnormal behaviors decreased significantly after the animals were transferred from single- to pair-housing; the five females had engaged in self-biting behavior when they were single-caged; all of them stopped this behavioral pathology once they were living with a companion.

Roberts & Platt (2005) studied one adult male long-tailed and eight adult male rhesus macaques who all had cranial implants and lived with compatible partners in a pair-housing arrangement. The presence of a social partner did not cause any problems with the implants, which lasted for an average of 21 months. Partners were separated daily for a few hours to participate in physiological experiments; this had no adverse effect on their compatibility which, depending on the length of the study, was confirmed for up to 40 months.

Murray et al. (2002) demonstrated the practicability of post-operative pair-housing in 15 female long-tailed macaques who were returned to their partners on the day of the operation. Change in dominance status, self-traumatic events, weight loss or diarrhea did not occur in any of these animals, and the incision sites healed unremarkably. The animals ate and drank normally, and they accepted their post-operative oral medication.

Coe & Rosenblum (1984) observed five adult male **bonnet macaque** pairs on four different days during 15-minute sessions in the course of the first week after the pairs were established. Subjects groomed each other on average 29% of the time.

Reinhardt (1994c) monitored five adult female and three adult male **stump-tailed macaque** pairs, who had lived together for 6 months, each pair for 60 minutes. On average, female partners groomed each other 19% and hugged each other 6% of the time; male partners groomed each other 13% and engaged in hold-bottom rituals 4% of the time.

Grooming-contact housing: Crockett et al. (1997) housed same-sex pairs of adult long-tailed macaques in double-cage units in which partners were separated 19 hours daily by a blind panel, and separated 5 hours daily by grooming-contact bars, allowing them to reach through with their arms. Of 16 female pairs tested, 100% were compatible and partners spent about 43% of scan sampling time grooming each other. Of 45 male pairs tested, 89% were compatible and partners spent about 7% of the time grooming each other.

The usefulness of grooming-contact bars, or woven wire panels with mesh openings large enough so that adjacent neighbors can groom each other (Coelho & Carey, 1990), has also been confirmed in adult heterosexual pairs of pig-tailed macaques (Crockett et al., 2001; Lee et al., 2005) and adult isosexual and heterosexual pairs of baboons (Coelho et al., 1991; Crockett & Heffernan, 1998). De Villiers & Seier (2010) transferred a single-caged subadult male baboon, who suffered from serious self-injurious biting, in a contact-grooming housing arrangement to a group of compatible females. The protected social contact resulted in healing of the self-inflicted laceration within 4 months. After 18 months, neither the self-injurious biting nor the wounds reoccurred.

Compared to other nonhuman primate species, rhesus macaques do not adjust well to the grooming-contact housing system (Crockett et al., 2006).

Social buffer: The compatible companion can serve as a social buffer during potentially stressful research-related situations, such as being transferred to a test room.

Coelho et al. (1991) measured blood pressure via arterial catheter implants of four tethered adult male **baboons** who were kept in an unfamiliar test room alone or in company of a familiar male baboon with whom they had visual, tactile and auditory contact through a wire mesh panel. Mean blood pressures were significantly lower when another baboon was present, suggesting that companionship mitigated the distress response (anxiety) to the test room environment.

Gust et al. (1994) transferred seven adult female **rhesus monkeys** from their group to an unfamiliar environment, either alone or together with a group member. During both conditions, the animals were initially equally distressed, as measured in alterations of cell-mediated immune parameters, but they recovered significantly quicker when they had the social support of a companion. Mason (1960) placed five infant rhesus macaques into a test room, either alone or as a pair with another infant. They showed significantly fewer signs of distress (crouching, self-clasping, vocalization, agitation) when they were tested in the company of another monkey, indicating that the companion had a calming, reassuring influence. Gunnar et al. (1980) confirmed these findings in 12 rhesus infants.

Similar observations were made by Hennessy (1984) in eight infant **squirrel monkeys** who vocalized significantly less when they were tested in an unfamiliar environment as a pair than when they were tested alone. A significant elevation of plasma cortisol was observed when the animals were exposed to the novel environment alone, not when a companion was present. Coe et al. (1982) noticed the same stress reducing effect in 14 adult male squirrel monkeys. Subjects showed significantly fewer distress reactions (vocalization, fear reactions, agitation) to a snake behind a mesh when another male was with them than when they were alone.

Recommendations: Compatible social companionship is probably the most important factor that influences the well-being of a nonhuman primate in the research laboratory. Unless there are veterinary and ethological reasons for exemptions, no nonhuman primate should be housed alone. Ideally, all animals should be housed with a companion, and at minimum, they should spend the whole night and most of the day with a companion. Animal care committees must be well informed about possibilities of allowing nonhuman primates to keep full or partial contact with another compatible companion during scientific investigations. The fact that historical data have been collected from single-caged primates is not an acceptable excuse for keeping animals alone during current research projects.

Refinement » Friendly contact with humans

Friendly contact with humans provides high-quality environmental enrichment by promoting and fostering mutual trust relationships between human and nonhuman primates.

Nonhuman primates respond to friendly attention from individual caretakers and individual investigators by gradually developing affectionate relationships with them, and overcoming their conditioned fear and distrust of humans. Positive interaction with monkeys and apes is essential for the well-being of the animals, data validity, and ease of handling (Wolfle, 1987). The behavior of a nonhuman primate during procedures depends on the confidence he or she has in the handler. This confidence is developed through regular human contact and, once established, should be preserved (Home Office, 1989). Good relations between the animals and personnel are important for animals to reduce stress and for personnel to obtain safer working conditions. Personnel who have gained the trust of animals can more easily perceive abnormal behaviors and the animals are more likely to cooperate with them during research procedures, such as restraint and blood sampling (The Primate Research Institute, 2003).

Animals who have developed a trust relationship with attending personnel give the impression that they like human contact. This suggests that human contact can have a relaxing, tension-releasing effect on them. Gantt et al. (1966) reports of a female rhesus macaque who was petted by a person several times on two different days. On both days a significant decrease of the animal's heart rate was noticed during the petting sessions.

Koban et al. (2005) exposed four male long-tailed macaques of unspecified age to daily 10-minute positive reinforcement training sessions for 2 months; four control subjects received no training sessions. The results indicate that the positive interaction with the human trainer made the animals feel at ease: serum cortisol concentration and heart rate were significantly lower in trained than in control subjects.

Baker (2004) increased the time from 2 to 4 hours that caretakers spent visiting—playing, grooming, talking, offering treats—seven adult female and five adult male chimpanzees housed in pairs and trios. Behavioral data were collected between the visits, allowing the carry-over effect of human interaction to be assessed. When the daily time of unstructured affiliation with personnel was doubled, the chimpanzees were more relaxed, spending more time grooming each other ($p<0.05$) and less time engaging in agonistic displays ($p<0.06$).

Recommendations: The quality of care provided by research personnel has a profound effect on the well-being of the animals and the quality of the science. Whenever possible, unstructured time should be set aside for personnel to spend time with the animals in their charge, so that human-animal interactions will be based upon affection and trust rather than apprehension, anxiety, and fear.

Refinement » Training to cooperate with humans during procedures

A friendly human-animal relationship based on mutual trust is the basic condition to obtain the cooperation of nonhuman primates during procedures that would otherwise require involuntary restraint and incur distress for the animal and risk for the human handler.

Training nonhuman primates to cooperate—rather than resist—during procedures achieves two goals at the same time:

1. Intellectual stimulation for the animal subject *and* for the human caregiver (enrichment);
2. Reduction of distress reactions of the animal and increase in safety of the personnel during husbandry- and research-related procedures (Refinement).

It has been documented for several species of nonhuman primates how individuals of both sexes, different age classes and different housing arrangements can be successfully trained to voluntarily cooperate with the attending personnel during the following research- and husbandry-related procedures:

Injection:

» single-caged adult male mandrill (Priest, 1991);
» single-caged adult male baboon (Levison, 1994),
» single-caged adult male mustached guenon (Stringfield & McNary, 1998);
» group-housed adult male lion-tailed macaques (Bayrakci, 2003);
» single-caged male squirrel monkeys (Gillis et al. 2012;
» single-caged, pair- and group-housed female and male chimpanzees of all age classes (Spragg, 1940; Videan et al.; 2005a; Russell et al., 2006) who required a mean total of about 87 minutes per animal to achieve the goal of the training.

Bentson et al. (2003) compared the stress response to injection in four single-caged rhesus macaques who were not trained with that of 17 single-caged rhesus macaques who had been trained to cooperate during this procedure. While serum cortisol concentrations did not increase in the trained subjects, cortisol increased significantly in the untrained subjects.

Blood collection:

» single-caged adult male mandrill (Priest, 1990);
» pair-housed adult female stump-tailed macaques who needed less than a cumulative mean total of 60 minutes per animal to achieve the goal of the training (Reinhardt & Cowley, 1992);
» single-, pair- and group-housed chimpanzees of both sexes and all age classes (Laule et al., 1996; Schapiro, 2000 & 2005; Coleman et al., 2008);
» single-caged and pair-housed rhesus macaques of both sexes and all age classes (Elvidge et al., 1976; Vertein & Reinhardt, 1989, Reinhardt, 1991b; Phillippi-Falkenstein & Clarke, 1992). Depending on the technique applied, a cumulative mean total of 40 to 160 minutes were invested to achieve the goal of the training (Reinhardt, 1991b; Pranger et al., 2006; Schapiro et al., 2007).

It has been shown in rhesus and stump-tailed macaques as well as in baboons that successfully trained animals show no behavioral and no physiological stress response—as measured in changes in serum cortisol concentration (Elvidge et al., 1976; Reinhardt, 1991b; Reinhardt & Cowley, 1992; Bentson et al., 2003)—when they cooperate during blood collection.

Blood pressure measurement:

» group-housed adult female and male woolly monkeys (Logsdon, 1995);
» single-caged adult male baboons (Mitchell et al., 1980; Turrkan et al., 1989).

Urine collection:

» group-housed adult male vervet monkeys (Kelly & Bramblett, 1981);
» group-housed adult female white-faced sakis (Shideler et al., 1994);
» single-caged and group-housed juvenile and adult chimpanzees (Laule et al., 1996; Lambeth et al., 2000);
» group-housed juvenile and adult marmosets of both sexes (Anzenberger & Gossweiler, 1993; McKinley et al., 2003; Smith et al., 2004);
» group-housed adult female tamarins (Snowdon et al., 1985; Smith et al., 2004).

Vaginal swabbing:

» group-housed stump-tailed macaques (Bunyak et al., 1982). After five training sessions of unspecified duration it was no longer necessary to net and restrain the females. Indeed, some of them began to voluntarily approach the researcher and present for vaginal swabbing.

Semen collection:

» group-housed gorillas (Brown & Loskutoff, 1998);
» group-housed chimpanzees (Perlman et al., 2003).

Oral drug administration:

» group-housed adult cotton-top tamarins of both sexes (Savastano et al., 2003);
» single-caged adult male baboons (Turrkan et al., 1989);
» single-caged and group-housed adult marmosets of both sexes (Peterson et al., 1988; Donnelly et al., 2007);
» single-caged adult male and females rhesus macaques (Winterborn, 2007; Anonymous, 2013).

Saliva collection:

» single-caged adult male rhesus macaques (Lutz et al., 2000);
» single-caged adult male squirrel monkeys (Tiefenbacher et al., 2003);
» single-caged and group-housed adult marmosets of both sexes (Cross et al., 2004);
» group-housed young baboons (Pearson et al., 2008).

Topical treatment:

» group-housed adult female gorillas (Segerson & Laule, 1995);
» group-housed female and male chimpanzees of all age classes (Perlman et al., 2001);
» pair-housed adult stump-tailed macaques of both sexes (Reinhardt & Cowley, 1990).

Weighing:

- pair-housed adult marmosets (McKinley et al., 2003); a cumulative mean total of about 1 hour per pair was needed to achieve the goal of the training.

Chairing:

- single-caged male pig-tailed macaques of unspecified age (Nahon, 1968);
- single-caged adult long-tailed macaques of both sexes (Skoumbourdis, 2008);
- single-caged juvenile and adult rhesus macaques of both sexes (Skoumbourdis, 2008); Bliss-Moreau et al., 2013):
- pair-housed juvenile rhesus macaques of both sexes (McMillan et al., 2014).

Capture:

- groups of bonobos (Bell, 1995);
- groups of Japanese macaques (Goodwin, 1997);
- groups of chimpanzees (Kessel-Davenport & Gutierrez, 1994; Boomsmith et al. 1998;
- groups of rhesus macaques (Reinhardt (1990c). In order to train a heterosexual group of 45 rhesus macaques to voluntarily cooperate during the routine one-by-one capture procedure, an average of 20 minutes was invested per group member, 15 hours for the whole group. It took about 15 minutes to catch all 45 animals without distressing them (Luttrell et al., 1994).

Checking faucet waterers: Checking automatic waterers can be time-consuming and cumbersome for staff, as well as intimidating for animals. Habe Nelsen et al. (2010) alleviated this by training eight rhesus and seven long-tailed macaques, using a laser pointer as the target, to check their Lixits. Seven of the rhesus and six of the long-tailed macaques were successfully trained; after several months, most of them longer needed the laser and checked the Lixits upon a verbal command and a simple hand gesture (Ferraro et al., 2013).

Recommendations: "Primates dislike being handled and are stressed by it; training animals to cooperate should be encouraged, as this will reduce the stress otherwise caused by handling. Training the animals is a most important aspect of husbandry, particularly in long-term studies. … Training can often be employed to encourage the animals to accept minor interventions, such as blood sampling" (Council of Europe, 2006, p 48). "Positive reinforcement techniques should be used to train primates to cooperate with capture, handling, restraint and research procedures. The routine use of squeeze-back cages and nets should be actively discouraged" (National Center for the Replacement Refinement Reduction of Animals in Research, 2006, p 11). Training nonhuman primates to cooperate rather than resist during husbandry and common research procedures is perhaps the most effective approach to minimize or avoid data-biasing stress reactions and, hence, reduce the number of subjects needed to achieve statistical significance of the research results (cf., Brockway et al., 1993; Schnell & Gerber, 1997; National Center for the Replacement Refinement Reduction of Animals in Research, 2006). Research laboratories need to make more earnest and more consistent use of the animals' amazing potential to work with rather than against principal investigators and animal care personnel during handling and husbandry procedures.

Refinement » Feeding enrichment

Feeding enrichment promotes noninjurious food searching, food retrieving and/or food processing activities.

Vegetables and fruits: The following unprocessed produce has been fed to captive primates without any adverse side effects: apples, oranges, bananas, grapes, watermelons, pumpkins, squash, potatoes, carrots, string beans, corn on the cob, lettuce, celery, artichokes, bell peppers, sugar cane, cranberries, raspberries, coconuts, and peanuts in the shell (Bloomsmith et al., 1988; Spector & Bennett, 1988; Hayes, 1990; Beirise & Reinhardt, 1992; Nadler et al., 1992; Williams et al., 1992; Logsdon, 1994; Waugh, 2002).

When presented behind a barrier—for example behind the bars or mesh of the enclosure—whole fruits and vegetables promote not only food processing, but also skillful food retrieval behavior.

Beirise & Reinhardt (1992) distributed every week 1 kg whole peanuts and on a different day 32 ears of corn to a 16-member breeding group of rhesus macaques. After a habituation period of 8 weeks, 120-minute observations were conducted immediately after peanuts or corn were distributed in weeks 9, 10 and 11. Individual animals spent on average:

- » 77% of the time husking corn ears, chewing husks, and eating corn kernels, and
- » 47% of the time cracking peanut shells and eating peanuts.

Recommendations: Attending care personnel typically work under time pressure. To have them chop supplemental vegetables and fruits for the animals can be quite time-consuming and is unnecessary. The animals have all the time needed to process the material themselves, and they like to do it. Offering whole, rather than already processed, vegetables and fruits of the season provides effective feeding enrichment without extra time investment. It introduces variety into the monotonous standard feeding regimen of commercial, pelleted dry food and allows the animals to engage in species-typical food processing behaviors. The provision of fruits and vegetables are not extra "treats" but a part of the standard food ration; every animal should have access to at least one medium-size whole fruit or vegetable on a daily basis.

Standard food ration behind a barrier: Offering the daily food ration not freely accessible on the floor or in standard food boxes, but behind the bars or mesh wall/ceiling of the enclosure, is probably the easiest way of increasing the time that the animals can spend retrieving and processing their food. Reinhardt (1993a) distributed the daily biscuit ration of eight adult male, pair-housed rhesus macaques (a) first in their ordinary, freely accessible food boxes, and then (b) for a 2-week period on the cages' mesh ceilings with 2.2 cm^2 openings. Time spent retrieving biscuits was recorded for each animal during 4 hours following food distribution.

- » When the ration consisted of 66 small, bar-shaped biscuits, average foraging time increased 80-fold, from 17 seconds to 1,363 seconds.
- » When the ration consisted of 32 large, star-shaped biscuits, average foraging time increased 296-fold, from 12 seconds to 3,551 seconds.

Working for their daily biscuit ration did not affect the males' body-weight balances.

Reinhardt (1993b,1993c) observed eight pair-housed, adult male rhesus macaques and five female and seven male single-caged adult stump-tailed macaques, each individual for 30 minutes after their daily biscuit rations were distributed either in the ordinary food boxes with 7.3 x 4.7 cm access holes or in the same boxes remounted onto the 2.2 cm^2-opening mesh front panels of the cages a few centimeters away from the original access holes. All animals were habituated over a 30-day period to receiving their food in the food puzzles; their body weights did not change in the course of that time period.

- Rhesus macaques spent, on average, less than 1 minute collecting biscuits from the food box versus 18 minutes retrieving them from the food puzzle.
- Stump-tailed macaques also spent, on average, less than 1 minute collecting biscuits from the food box versus 19 minutes retrieving them from the food puzzle.

Bertrand et al. (1999) report of four single-caged rhesus macaques, of unspecified age and sex, who received their daily pellet ration in a freely accessible standard feeder, and four other single-caged subjects who received their pellet ration on 4 days in a foraging device fitted on the front of the cage. Manipulative skills were required to retrieve the pellets from this device. Over 90% of the food was eaten within the first 15 minutes with the standard feeder, whereas it took 60 minutes to reach this percentage using the foraging feeder. The amount of food waste was up to 17 times lower when the animals had to work for their food instead of collect it freely.

Murchison (1995) videotaped the behavior of 20 single-caged adult female pig-tailed macaques, each animal for 1 hour, when the ration of 40 biscuits was presented in the standard feeder with one big access hole (5 cm diameter) versus the same feeder with four small access holes (3 cm diameter). The animals spent, on average, 7 minutes using hands, teeth and feet to remove biscuits from the feeder with small holes versus less than 1 minute to collect biscuits from the standard feeder with one big access hole; the difference was statistically significant. Unlike with the standard feeder, the animals consumed most of the biscuits they retrieved from the test feeder; this implied that they dropped fewer pieces of biscuits on the floor and less food was wasted.

Bloom & Cook (1989) mounted a commercial puzzle feeder on the front panel of the cages of two adult male rhesus macaques and habituated the animals to retrieving their daily single portion of biscuits from this device. It took the two males, on average, 25 minutes to retrieve their food.

Expanded feeding schedule: Taylor et al. (1997) expanded the feeding schedule of a group of four adult female and one adult male bonnet macaques by dispersing one-half of the daily ration of 150 biscuits and 1 cup of sunflower seeds on the woodchip litter at the usual time in the morning, and the other half in the afternoon. Over a period of 10 weeks, the animals were observed during several 10-minute sessions starting 1 hour after food distribution. When they received their daily food ration in two small portions (weeks 6-10), rather than in one big portion (weeks 1-5), they spent 52% versus 26% of the observation periods foraging.

Special food in and on gadgets: Numerous devices, baited with food treats rather than the standard food have been developed to encourage foraging-related activities in captive primates.

Brent & Eichberg (1991) attached one Plexiglas sheet with holes on the mesh ceilings of the enclosures of eight heterogeneous groups of three or four **chimpanzees**. After a 7-day habituation period, commercial food treats were placed on these puzzle boards on four different occasions and the animals' response was recorded. During 1-hour observation sessions the chimpanzees spent, on average, 17% of the time retrieving treats from the puzzle.

Maki et al. (1989) designed metal pipe-feeder puzzles containing sticky foods—such as applesauce, mashed bananas, spaghetti sauce, and dry fruit drink powder. Four adult chimpanzees, living with other companions in pairs or trios, were observed during eight 30-minute sessions distributed over a period of 1 month, when a daily-filled pipe feeder was permanently mounted from outside on the chain-link fencing of the home quarters. The four subjects spent, on average, 23% of the sessions manufacturing dipping sticks from branches, and an additional 29% fishing with these tools for the moist foodstuff in the box.

Celli et al. (2003) mounted an open transparent polyethylene bottle, which was filled daily with honey, in front of the cages of three pairs of adult female chimpanzees and offered them plastic brushes, wires, chopsticks and rubber tubes from which they could chose suitable tools for retrieving honey from the bottle. During daily 60-minute observations—probably right after the bottle was filled—individual animals spent about 9 % of the time checking out suitable fishing tools, and 31% of the time retrieving honey from the bottle.

Gilloux et al. (1992) monitored a heterogeneous group of seven chimpanzees for twelve 120-minute sessions when a 15-cm-diameter plastic pipe filled with fruits, vegetables and biscuits was attached outside onto the welded mesh of the enclosure. The apes could manipulate food items to the open end of the pipe by inserting bamboo canes or willow twigs through holes drilled along the side of the pipe facing them. Individuals used the filled feeder, on average, during 18% of the time.

Lambeth & Bloomsmith (1994) conducted six 30-minute observations of eight adult female and six adult male chimpanzees, living in pairs or groups of four, after a PVC pipe cut in half and planted with rye grass was attached to the front panel of the chain-link fencing of the subjects' enclosures. Individual animals spent, on average, 4% of the time picking grass with their fingers through the fencing; when sunflower seeds were added to the grass, they spent 20% of the time searching for and picking up seeds.

Bayne et al. (1992) secured Plexiglas boards covered with artificial turf inside the cages of eight single-housed adult male **rhesus macaques**. Commercial, flavored food particles were sprinkled on the turf boards daily 2 hours after the morning feeding; this was followed by 30-minute observations of each subject on 20 days over the course of a 6-month period. The males foraged, on average, during 52% of the observation periods; there were no signs that they lost interest in foraging from the turf boards over time.

Lutz & Farrow (1996) mounted turf boards to the outside of the front panel of the cages of ten adult female **long-tailed macaques** and sprinkled sunflower seeds on the turf every morning, after the animals had received their daily biscuit ration. During three weekly 30-minute observations conducted at random times over a period of 8 weeks, the animals spent an average 11% of the time contacting the board. The boards were used by the animals with consistency; there was no indication that they lost interest in them over time.

Bryant et al. (1988) released six individually caged, adult male long-tailed macaques, one animal at a time, for 30 minutes into a playpen on 12 days, distributed over a 3-week period. The playpen was furnished with a nylon ball, a telephone directory, a nylon rope and a tray placed below the grid floor of the cage, containing woodchips scattered with sunflower seeds and peanuts. The animals showed little interest in the nonfood enrichment items but spent a considerable amount of the time reaching through the wire mesh of the cage floor to retrieve seeds and peanuts.

Fekete et al. (2000) mounted a turf board inside, on a shelf of the cages of 10 pair-housed adult female **squirrel monkeys** and sprinkled a mixture of nuts, seeds and dried fruits onto the board on 11 consecutive days, right after the normal food was distributed. During the first 20 minutes, individuals spent approximately 36% of the time foraging.

Chamove & Scott (2005) made 360-minute video recordings of four family groups (5–11 individuals) of cotton-top **tamarins** after they were presented with a forage box to which they were habituated. The box was filled with a mixture of sawdust and small food items. Over the 6 hours, any given monkey was engaged in searching for and retrieving food from the box approximately 7% of the time.

Roberts et al. (1999) injected acacia gum into 2.5-cm-deep holes of 30-cm-long branch segments and placed one gum feeder each in the cages of 28 adult **marmosets**. The feeders were left in the cages for 5 days and the animals tested right after gum was injected into the branch on day 1 and day 5. During the 30-minute test sessions, individual animals spent, on average, 43% of the time gum-foraging on day 1, and 10% of the time gum-foraging on day 5. The branches were already heavily gouged on day 5.

Special food mixed with a substrate: Anderson & Chamove (1984) observed eight group-housed young stump-tailed macaques who were kept on woodchip litter (a) during 2 days before, and (b) during 2 days after 350 grams of mixed grain was distributed on the litter. In the course of 220 one-minute scan sessions conducted in both conditions, individual animals spent, on average, 6% of the time foraging on plain litter versus 30% of the time foraging on litter mixed with grain. Blois-Heulin & Jubin (2004) studied red-capped mangabeys and reported similiar findings.

Bryant et al. (1988) observed six adult male long-tailed macaques alone in a test cage each day for 30 minutes. The cage had a tray placed below the grid floor containing woodchips mixed with sunflower seeds and peanuts. Individuals spent approximately 37% of the time reaching through the grid floor, searching for and retrieving food from the woodchip litter. The interest in this activity increased over the course of a 12-day study period.

Recommendations: Nonhuman primates are biologically programmed to spend a major portion of their time foraging: searching for, retrieving and processing food. Allowing them to engage in foraging activities, rather than pick up freely accessible food in the research laboratory setting is an easy option for environmental enrichment; it should be a default practice in every primate research institution. The least expensive, yet very effective feeding enrichment is the distribution of the daily ration in such a way that the animals have to engage in skillful manipulation techniques to retrieve and process the food.

Refinement » Inanimate enrichment

Inanimate enrichment increases the complexity of the living quarters and promotes noninjurious contact and interaction with objects.

Structural enrichment » perches: The spatial limitation of the legally minimum-size standard cage can make it quite a challenge to open up the vertical dimension for the confined animal in a species-appropriate manner. This applies particularly for animals caged in the lower row of the prevailing double-cage arrangements. These animals are forced to live in a shady, cave-like environment close to the ground. Not surprisingly, when given the choice to stay in a bottom-row or in a top-row cage, macaques show a strong preference for the upper row cage (Westlund, 2002; MacLean et al., 2009).

A high perch opens up the vertical dimension, thereby increasing the usable cage space and promoting species-adequate behaviors, such as climbing, leaping (if the cage is large enough), balancing, bouncing, perching, sleeping, looking out, retreating to a safe place during alarming situations, and retreating to a dry place during the cage-cleaning procedure. Access to a high resting site has survival value for nonhuman primates. This explains why they do not lose interest in high resting surfaces over time.

Reinhardt (1989) assessed the time budgets of 25 adult male rhesus macaques who were housed in single-cages, each equipped with a 120-cm-long polyvinyl chloride (PVC) pipe that had a diameter of 5 cm and was installed diagonally, with a slope of 15°, about 40 cm above the floor. The males had been exposed to these perches for 12 months. There were 14 males in upper-row cages and 11 males in lower-row cages. During 120 minutes of observations, the average amount of time spent on the perch was:

- » 45% for the males in lower-row cages, versus
- » 15% for the males in upper-row cages; the difference was statistically significant.

Lower-row cage individuals were probably more attracted by their perch because they lived closer to the ground and at a greater distance from the light source. Sitting on an elevated surface was more advantageous for them than for individuals in the high and relatively bright upper-row cages.

Woodbeck & Reinhardt (1991) confirmed these findings in 28 pairs of adult female rhesus macaques who lived in double cages, each furnished with two 120-cm-long PVC pipes, located either in the bottom row (n=14 animals) or in the top row (n=14 animals). The females had been exposed to these perches for more than 24 months. During seven 30-minute observations conducted in the late afternoon when personnel were no longer in the building, average amount of time spent on the perch was:

- » 33% for the females in lower-row cages, versus
- » 7% for the females in upper-row cages; this difference was also statistically significant.

Similar findings were reported by Shimoji et al. (1993), who attached four parallel-connected PVC pipes, 5 cm in diameter, to the back of the cage 27 cm off the floor, of 10 female and 10 male adult long-tailed macaques for a 3-day study period. Remote video recordings revealed that animals caged on the bottom row of the rack spent, on average, 26% of the day on the perch, while animals caged on the top row spent only 14% of the day on the perch; the difference was not statistically different, but it was consistent on each of the 3 days.

Elevated structures not only make the vertical dimension accessible to the animals, but they also provide them with easy ways of quickly getting away from each other in situations of potential antagonistic conflict. Kitchen & Martin (1995) observed five pairs of common marmosets, each for a total of 20 hours, when their cages were barren versus equipped with three perches, 2.5 cm in diameter. When they had access to perches, the marmosets stopped showing startle responses and the incidence of aggressive interaction was significantly reduced. Neveu & Deputte (1996) recorded the behavior of a breeding troop of gray-cheeked mangabeys, consisting of three adult and two juvenile females and one adult and one subadult male, during 30-minute sessions when they lived in a barren cage versus a cage of the same dimensions but fitted with four perches at different heights. Access to perches decreased agonistic behaviors from about 25% to 0 % of all interactions; at the same time it increased socially positive behaviors significantly from about 2% to 10% of all interactions. Nakamichi & Asanuma (1998) tested a group of four adult female Japanese macaques in two identically sized enclosures that were either unstructured or furnished with eight wooden perches at different heights. Several 15-minute observation sessions showed that the average number of agonistic interactions was significantly lower in the furnished cage than in the unfurnished cage.

Structural enrichment » swings: In their natural habitat, nonhuman primates usually do not swing on branches or lianas. It is, therefore, not surprising that they have little use for swings in research labs, especially since the small size of their living quarters does not provide sufficient space to actually swing back and forth.

Bryant et al. (1988) observed six single adult male long-tailed macaques daily for 30-minutes in a play cage that was equipped with a swing suspended 60 cm from the ceiling. During a period of 12 days, two males never used the swing; the four others spent, on average, less than 2% of the time on it.

Kopecky & Reinhardt (1991) installed a PVC perch in one section and a PVC swing at the same height in the other section of upper-row double cages of 14 adult, pair-housed rhesus macaques and observed each animal after 1 month for 60 minutes. Subjects spent, on average, 7 minutes on the perch, but only half a minute on the swing. It was concluded that the animals' statistically significant preference for the perch was probably related to the fact that the perch, unlike the swing, was a fixed structure permitting continuous relaxed postures rather than brief balancing. Moreover, the perch, unlike the swing, allowed the animals to sit right in front of the cage within sight of the events going on in the room.

Dexter & Bayne (1994) tested nine adult single-caged rhesus macaques of both sexes in the presence of either two types of PVC swings, a hemp rope swing or a swing made of artificial vine. Each animal was exposed to the swings for a three-week period and observed three times for 30-minute sessions. The animals manipulated the swings but showed little inclination to actually use them for swinging. Altogether, swinging was witnessed only six times in the course of 360 minutes of observation; the average time that a monkey was actually swinging was less than 1 minute.

Also in relatively large group-enclosures, adult primates show hardly any interest in movable structures such as swings, ropes, suspended barrels or Ferris wheels, but they will spend most of the day and all night on fixed structures such as platforms, shelves, ladders, benches, and perches well above the floor area (langurs: Schwenk, 1992; rhesus macaques: Lehman & Lessnau, 1992; baboons: Kessel & Brent, 1996; chimpanzees: Howell et al., 1997).

Structural enrichment » visual barriers:

The spatial constraint of the cage makes it difficult to add structures in which an animal can take visual refuge from a dominant cage mate, but vertical blinds can readily be installed without occupying part of the floor area.

Reinhardt & Reinhardt (1991) inserted a privacy panel, consisting of a sheet of stainless steel with a rectangular 23 x 32 cm large passage hole close to the back wall of the cage, between the two halves of each double cage of 15 adult female rhesus pairs. One-hour observations before, and 7 days after placement of the privacy panels revealed that companions:

- spent significantly more time in the same half of the cage (46 versus 37 minutes),
- spent significantly more time engaged in affiliative interactions (22 versus 16 minutes), and
- had fewer agonistic disputes (0.3/h vs. 2.2/h; difference statistically not different) when they had the option of visual seclusion.

Basile et al. (2007) observed 18 male/male pairs, 2 female/female pairs, and 5 male/female pairs before and 1 week after a privacy divider was placed in their double cages. The blind was oriented in such a way as to physically divide the front half of the cage, while leaving open access through the rear half. With the privacy divider in place, the animals spent significantly more time in the same half of the cage than without the divider. It was concluded that the privacy divider may provide a safe haven and give monkeys the ability to diffuse hostile situations before they escalate. McCormack & Megna (2001) placed privacy panels into the enclosure of a 126-animal breeding troop of rhesus macaques and noted a significant decrease in threatening, chasing, fear grinning, and screaming. Estep & Baker (1991) observed a breeding troop of 26 stump-tailed macaques during 90-minute sessions both before and after two solid temporary walls were erected within the animals' enclosure. The incidence of contact aggression was significantly lower when the monkeys had the option of breaking visual contact with other group members by moving behind these walls. Maninger et al. (1998) installed visual barriers in the living quarters of two breeding groups of 23 pig-tailed macaques and noted that the option of visual seclusion significantly reduced instances of biting, grabbing and chasing.

Erwin et al. (1976) studied agonistic interactions between adult female pig-tailed macaques who lived in four breeding groups; there were approximately 12 females in each group. Daily 20-minute observations were conducted of each group (a) during a 5-day control period and (b) during a 5-day experimental period when a concrete cylinder, approximately 1 m in length and 50 cm in diameter, was firmly placed in each enclosure. The mean incidence of agonistic interactions per 20-minute observation session was 94 during the control condition versus only 45 during the experimental condition; the difference was statistically significant. The monkeys used the cylinders as escape routes to hide from potential aggressors.

Recommendations: Nonhuman primates spend the night and a great portion of the day on elevated sites at a safe distance from terrestrial threats. "Even macaques, which some describe as semiterrestrial, spend most of the day in elevated locations and seek the refuge of trees at night. These animals might perceive the presence of humans above them as particularly threatening" (National Research Council, 1998, p 92 & 118). It is very important that their enclosures in research facilities are furnished with elevated structures allowing the animals to retreat to and rest on relatively safe surfaces. The usefulness of such structures depends on their placement in the cage. Any resting surface should be installed in such a way that an animal can:

- sit right in front of the cage and check out what is going on in the room,
- retreat to the back of the cage when frightened, for example, when a fear-inducing investigator enters the room,
- sit on it without touching the ceiling with the head and without touching the floor with the tail, and
- use the space beneath it for normal postural adjustments.

High resting surfaces are not really enriching the environment of the animals; they are a *necessity* and, therefore, should be mandatory furniture in every primary enclosure of nonhuman primates.

Visual barriers and/or safe escape options—with entrance *and* exit—should be mandatory provisions of any primary living quarters of pair-housed and group-housed primates in order to minimize conflicts triggered by the unnatural spatial constraint of confinement.

Toys: Nonhuman primates are too intelligent not to quickly get bored by toys, unless these can gradually be destroyed. Not surprisingly, they are much more interested in destructible than in durable toys (chimpanzee: Shefferly et al., 1993; Brent & Stone, 1998; Videan et al., 2005b; orangutan: Heuer & Rothe, 1998; pig-tailed macaques: Cardinal & Kent, 1998).

A conspicuous habituation to most commercial toys has been documented in chimpanzees (rubber and plastic toys for small children: Paquette & Prescott, 1988; Kong toys: Pruetz & Bloomsmith, 1992; indestructible toy ball: Shefferly et al., 1993), rhesus macaques (nylon balls: Ross & Everitt, 1988; plastic toys for small children: Hamilton, 1991; nylon balls and rings, Kong toys: Weick et al., 1991; Kong toys: Bayne et al., 1993), baboons (nylon bones: Brent & Belik, 1997), long-tailed macaques (Kong toys: Crockett et al., 1989) and pig-tailed macaques (plastic toys for small children: Cardinal & Kent, 1998; rubber and rawhide balls: Kessel & Brent, 1998). To be of some value for the animals, most commercial toys need to be replaced on a regular basis to make use of their short-lived novelty effect.

Gnawing sticks: Unlike many commercial toys, dry deciduous tree branches cut into gnawing sticks do not lose their novelty effect over time, since they steadily change their configuration and texture due to wear and progressive dehydration. The animals use the sticks for gnawing, nibbling, chewing, manipulating and playing. Long-term use of gnawing sticks by several hundred rhesus macaques resulted in no recognizable health hazards (Reinhardt, 1997a).

Reinhardt (1990d) had provisioned 20 adult pair-housed stump-tailed macaques each with gnawing sticks for 2 months. During a 60-minute observation session, 16 of the animals gnawed the wooden material, on average, 8% of the time.

Reinhardt (1990a) assessed the time budgets of 60 pair-housed rhesus macaques of both sexes. Each pair had continuous access to one regularly replaced gnawing stick for 18 months or longer. During two 30-minute remote video recordings, the gnawing stick was used by 94% (17/18) of the subadult animals versus 64% (27/42) of the adult animals. On average, subadults spent 10% and adults spent 3% of the recording time in direct contact with the stick. The sexes did not differ significantly in their use of the wooden sticks; these were not only gnawed but also carried around and manipulated.

Sticks of sun-dried red oak branches are particularly suitable because they gradually wear into flakes that are so small that even large quantities pass through the sewer drains without clogging them (Reinhardt, 1992).

Paper and cardboard boxes: Recycled paper and cardboard boxes are not expensive, but they can offer effective environmental enrichment for primates in small cages or larger enclosures.

Kessel et al. (1995) scattered shredded paper once a week throughout the room of a group of five young male chimpanzees. After a habituation period of 1 week, 54-minute daily observation sessions were carried out during 2 weeks. The animals spent, on average, 27% of the time playing with the paper.

Bryant et al. (1988) transferred six adult male long-tailed macaques from their standard home cages to a play pen, furnished with a telephone directory and a nylon ball, each day for 30 minutes over a 12-day test period. The animals had very little or no use for the nylon ball, but they spent, on average, 10% of the time examining and shredding the telephone directory. Their interest in the paper material remained fairly constant; there was no indication that they lost interest in it over the course of time.

Beirise & Reinhardt (1992) placed a cardboard box into the pen of a 16-member breeding group of rhesus macaques once a week. After a habituation period of 8 weeks, the animals were observed for 120 minutes after placement of the cardboard box during weeks 9, 10 and 11. Individuals spent, on average, 65% of the time with the box, tearing it apart, shredding it and chewing pieces of it.

Water: Basins filled with water for swimming, diving for food items, fishing for food items, and playing have been employed for caged and group-housed long-tailed macaques (Gilbert & Wrenshall, 1989), squirrel monkeys (King & Norwood, 1989), and rhesus macaques (Anderson et al., 1992; Rawlins, 2005) without adverse effects, other than much splashing.

Mirrors: Both apes and monkeys are fascinated by their own reflections, and they use a mirror to check out the immediate environment without directly looking at it (Gallup, 1970; Lethmate & Dücker, 1973; Eglash & Snowdon, 1983; Platt & Thompson, 1985; Anderson, 1986; Lambeth & Bloomsmith, 1992; O'Neill et al., 1997; Chiappa et al., 2004; de Waal et al., 2005; Schultz, 2006).

Mirrors that can be manipulated are particularly useful for animals who are housed alone, while socially housed animals tend to focus their attention more on the social partner than on the mirror. Harris & Edwards (2004) hung stainless steel, 15-cm-diameter mirrors on the cages' front panels of 25 single male vervet monkeys, and observed each subject during four 30-minute sessions, 10 months, and again 16 months after the initial introduction of the mirrors. The average amount of time spent contacting the mirror and looking into the mirror was consistent at 5%, indicating that the animals had a sustained interest in them.

Windows: Whenever possible, rooms housing nonhuman primates should be provided with windows, since they are a source of natural light and can provide health benefits as well as environmental enrichment (International Primatological Society, 2007).

Pairs of male long-tailed macaques, transferred regularly for 1.5 hours to a playroom with windows, spend about 67% of the time looking out the windows (Lynch & Baker, 2000).

Light: There seems to be an international regulatory and professional consensus that lighting must be uniformly diffused throughout animal facilities and *must* provide sufficient illumination to facilitate housekeeping, cleaning, and inspection of animals, and maintain the well-being of the animals (United States Department of Agriculture, 1991; cf., Institute of Laboratory Animal Resources, 1980; National Research Council, 1996; Fortman et al., 2002; International Primatological Society, 2007). These important stipulations are meaningless as long as the traditional double-tier caging system prevails in some countries (e.g., Rosenberg & Kesel, 1994). Sanitation trays beneath the upper tier of cages, reduce significantly the amount of light from ceiling-mounted fixtures that can penetrate to the lower-cage tier; animals in the lower tier are thus relegated to a permanent state of semi-gloom (Mahoney, 1992). Illumination is often so poor that flashlights are needed to identify animals, check their well-being and make sure that the floor and the corners of the cage are adequately cleaned (Reinhardt, 1997b; Reasinger & Rogers, 2001; Savane, 2008).

Rotating cage positions relative to the light source—as is sometimes recommended (Canadian Council on Animal Care, 1993; National Research Council, 1996) and practiced (Ott, 1974; Ross & Everitt, 1988; Shively, 2001; Buchanan-Smith et al., 2002)—rotates the inherent problem, but it does not solve it: There will always be half of a population of double-tier caged animals who live in the lower tier in the shade cast by the cages of the upper tier.

Recommendations: "A two-tiered system is not recommended as these cages are usually too small. The lower tiers do not allow primates to engage in their vertical flight response, are often darker, and animals in the lower cages tend to receive less attention from attending personnel" (International Primatological Society, 2007, p 12). Keeping nonhuman primates in single-tier, rather than multi-tier caging systems in tall cages equipped with high resting surfaces is, at the moment, the only satisfactory refinement option to deal with the problems associated with the lower-row cage situation. It:

- provides all animals of the room uniform illumination,
- creates uniform illumination to aid in maintaining good housekeeping practices, adequate cleaning, and adequate inspection of animals, and

» allows the animals to access the arboreal dimension of their enclosures and retreat to relatively safe vantage points above eye-level of attending personnel.

Wall-mounted lights illuminating lower-row cages from behind can possibly even out the illumination differences between upper and lower row (MacLean et al., 2009), but they will keep the occupants of the lower row cages restricted to the terrestrial dimension.

Videos, television and music: Schapiro & Bloomsmith (1995) presented 49 single-caged yearling rhesus macaques with videotapes of chimpanzees and rhesus macaques in natural settings most of the day for a period of 3 months. During 15-minute observation sessions, subjects were looking at the monitor about 7% of the time. The possibility was not ruled out that the animals would have shown the same interest in the blank monitor.

Markowitz & Line (1989) mounted a radio device on the cages of five single adult female rhesus macaques. The radio had been available for a 14-week period and was preset to a soft rock music station; the animals could turn the radio on and off by touching two different bars. When they were tested during weeks 8–14, individual animals turned on the radio for 0–24 hours per day; on average the radio was turned on for about 12 hours per day. The monkeys showed no signs of losing interest in listening to the music.

Brent & Weaver (1996) noted in four single-caged baboons that the animals' mean heart rate was significantly lower when they could listen to a radio station playing oldies than when the radio was turned off. This calming effect may have been indirect, with the music masking the noise coming from other animal rooms, the ventilation system, and the caretaking staff.

McDermott & Hauser (2007) gave four adult cotton-top tamarins and four adult common marmosets the choice of listening to various noises and various kind of music. The animals showed a significant preference for soft over loud noise and for slow-tempo over fast-tempo music. Both tamarins and marmosets strongly and consistently preferred silence over musical stimuli (flute lullaby: $p<0.0001$; sung lullaby: $p<0.003$; Mozart concerto: $p<0.0001$), suggesting that they did not find such stimuli pleasurable or relaxing.

Recommendations: Before an institution plans to implement video, television or music enrichment programs for its nonhuman primates, it is advisable to first check if the animals actually benefit from such an investment. Playing music, videos or television programs in animal rooms may entertain the attending personnel but not necessarily the caged animals; they may have different preferences—including silence—which need to be respected to safeguard the animals' well-being.

Conclusions

Species-adequate, effective and practicable options for providing social enrichment, feeding enrichment and inanimate enrichment, and practicable options of training nonhuman primates to cooperate during common procedures have been described, tested and documented in the scientific and professional literature. Making life easier for nonhuman primates in research laboratories is not only a very basic ethical responsibility of the biomedical research industry and an important animal welfare issue, but it is also a fundamental condition for the scientific validity of the research data collected from these animals (Animal Welfare Institute, 1979; National Research Council, 1985; Meyerson, 1986; Donnelley, 1990; Morton, 1990; Novak & Bayne, 1991; Schwindaman, 1991; Institute for Laboratory Animal Research, 1992; Chance & Russell, 1997; Fuchs, 1997; Öbrink & Rehbinder, 1999; Richmond, 2002; Reinhardt & Reinhardt, 2002; Russell, 2002).

"Animals should be housed with the goal of maximizing species-specific behaviors and minimizing stress-induced behaviors" (National Research Council, 1996, p 21-22). "The maintenance and use of nonhuman primates should only be permitted in facilities which can truly provide the high quality of housing, and care and attention which these animals require, if their normal physiology and behavior are to be maintained" (Balls, 1995, p 286).

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Extraneous Variables

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"A GOOD MANAGEMENT PROGRAM [for animals in biomedical research institutions] provides the environment, housing, and care that ... minimizes variations that can affect research" (National Research Council, 1996, p 21). Indeed, it would be naïve to rely on data collected from animals who experience fear and anxiety when they are approached by an investigator, from animals who suffer from depression and frustration resulting from the inability to show species-typical behaviors, and from animals who experience fear and anxiety resulting from involuntary restraint during procedures. In the context of biomedical research, these distressing experiences constitute unaccounted-for variables that affect the animals' physiological homeostasis. Sound scientific methodology requires that research data are not influenced by uncontrolled variables. Unless this requirement is met, an experiment is not considered scientifically valid (American Medical Association, 1992).

This chapter reviews common extraneous variables, discusses their potential impact on research data, and elaborates briefly on refinement options that minimize, eliminate, or avoid them.

Investigator

It seems to be a widely accepted practice among biomedical investigators to *use* animals as tools to promote their professional careers (Rollin, 1995). For the presumed sake of objectivity, animals are regarded as standardized test objects/models/systems rather than sentient beings endowed with feelings (Hummer, 1965; Arluke, 1988).

It is not uncommon that investigators have little or no direct contact with the animals of their research programs (Traystman, 1987; Arluke, 1993; Herzog, 2002; Baumans et al., 2007; Reinhardt, 2013); they are not aware when uncontrolled variables related to animal husbandry, care and handling are affecting the data obtained (Reese, 1991; Öbrink & Rehbinder, 1999; Baldwin, et al., 2007) and, therefore, require an unnecessarily large number of animals in order to achieve statistical significance of the research results (Russell & Burch, 1959; Home Office, 1989; Brockway et al., 1993; Baldwin et al., 2007; Reinhardt, 2013). Many investigators do not realize the influence of environmental variables on experimental results or at least do not adequately describe the environmental history of the animals used for experimentation (Davis et al., 1973; Claassen, 1994; Reinhardt & Reinhardt, 2000a; Reinhardt & Reinhardt, 2000b).

When handling animals during a research procedure, many investigators do it with little or no consideration that these animals experience anxiety, fear and distress—feelings that change their physiological equilibrium and, hence, influence the research data collected from them. Not giving much thought about how an animal feels during an uncomfortable and life-threatening procedure, it is perhaps not surprising that investigators often fail to describe how the animals were handled and/or how they reacted during the data collection procedure, even if stress-dependent parameters were measured (Reinhardt & Reinhardt, 2000a).

As a result of adverse conditioning, animals in research laboratories often show intense fear and physiological stress reactions when the researcher enters their room (Tatoyan & Cherkovich, 1972; Döhler et al., 1977; Manuck et al., 1983; Schnell & Wood, 1993; Späni et al., 2003; Reinhardt, 2013). The investigator may be completely unaware that he or she constitutes an overlooked extraneous variable in the research endeavor.

"Whether an investigator maintains a high personal respect for the well-being of the individual animal or holds classic concepts of animals as being experimental models, it should be more widely recognized that there is typically a scientific necessity to have animals at ease with their environments if studies are to remain objective" (Warwick, 1990, p 363). "Stressed animals do not make good research subjects" (American Medical Association, 1992, p 18).

Noise

Animals in research facilities are very often exposed to artificial noise without the option of retreating to a quiet location within their living quarters.

The noise environment of animals in research labs is usually not mentioned in the methodology section of scientific articles, even though it is likely to have important implications for the validity of data collected from such animals (Gamble, 1979; Clough, 1982; Milligan et al., 1993; Claassen, 1994). Noise as a potential data-confounding variable and a potential animal welfare concern has been given little consideration in the published literature. The few studies published do suggest that common sources of noise in the research laboratory are stressors that activate the hypothalamic-pituitary-adrenal axis.

Construction noise is an uncontrolled stressor for animals confined in cages. Mice and rats show a significant increase of plasma ACTH and corticosterone secretion along with a disruption of energy balance when they are exposed to construction noise and associated vibrations (Dallman et al., 1999; Raff et al., 2011). Exposure to construction noise can decrease the reproductive efficiency of guinea pigs (Anthony & Harclerode, 1959), mice (Rasmussen et al., 2009; Reinhardt, 2010), rats (Schipper et al., 2011), rabbits (Reinhardt, 2010) and zebrafish (Reinhardt, 2010).

The banging of metal cages in animal rooms can produce bursts of intense noise, triggering a significant endocrine stress response not only in rats (Barrett & Stockham, 1963) but probably also in other animal species found in research labs. Anecdotal evidence indicates that long-tailed and rhesus macaques get very agitated and resort to behavioral pathologies such as hair-pulling and self-biting when noisy construction work occurs nearby (Reinhardt, 2010). Marmosets show a significant rise in saliva cortisol concentrations during periods of loud noise caused by routine human activities (Cross et al., 2004) or by noisy construction work (Pines et al., 2004). A significant increase in fecal cortisol concentration was noticed in long-tailed macaques who were exposed to construction noise (Westlund et al., 2012).

Investigators may be unaware that exposure to common noises in animal holding areas affects the physiological equilibrium of animals and hence the quality of research data obtained from them (Jain & Baldwin, 2003; Baldwin et al., 2007). Noise in research animal facilities could be buffered with readily available industrial and architectural sound absorbing panels (Carlton & Richards, 2002; Jeans et al., 2008) along with sound buffering epoxy flooring (Johnson et al., 2005); noise levels could be reduced with considerate and correct working methods (Voipio et al., 2006); some noise sources—such as loud radio, loud talking, squeaky carts and squeaky doors—could be eliminated altogether.

Understimulation

The living quarters of animals in research facilities are often intentionally not provisioned with species-adequate stimuli, so that they are the same for all research subjects. The easiest way to create such *standardized* living quarters is to keep them empty of anything that is not necessary for the tenants' survival. It is not correct that "barren environments *may* not meet the species-specific needs of an animal [emphasis added]" (Committee on Recognition and Alleviation of Distress in Laboratory Animals, National Research Council, 2008, p 55); barren living quarters *cannot* meet these needs.

Animals kept in an impoverished environment with no or insufficient opportunities to perform genetically programmed, species-typical behaviors are unlikely to be good models for research (Hockly et al., 2002; Baldwin, 2010) because their physiological and behavioral response to chronic understimulation (boredom) will not be uniform. Some will cope reasonably well, others will get so frustrated that they get distressed and develop behavioral pathologies. A boring environment, therefore, bears the risk that not all subjects will respond to a test situation in a normative manner.

The most serious condition of understimulation is single housing of gregarious animals. In mice, distress resulting from social deprivation can decrease their resistance to spontaneously developing tumors (Andervont, 1944; Muhlbock, 1951), increase the variance of sensitivity to drugs (Mackintosh, 1962) and toxic agents (Hatch et al., 1965), alter immunological responses, brain neurochemistry, learning ability, and pain thresholds (Valzelli, 1973), produce abnormal behaviors and changes in body weight, lead to alterations of organ weights, blood cell counts, and adrenal function (Baer 1971), increase heart rate (Einstein et al., 2000), and disrupt the normal circadian sleep pattern (Späni et al. 2003). Individually caged rats show a compromised immuncompetence and, hence, an impaired resistance to disease (Ader & Friedman, 1964); they also exhibit a greater incidence of stereotypical tail manipulation and pawing (Baenninger, 1967; Hurst et al., 1997), a higher heart rate and blood pressure (Sharp et al., 2002a), higher levels of corticosterone and prolactin (Cambardella et al., 1994), higher variations in certain biochemical parameters (Pérez et al., 1997) and a lower survival rate than rats housed in groups (Shaw & Gallagher, 1984). When they are housed alone, guinea pigs markedly lose weight and reduce their water intake (Fenske, 1992).

Rabbits are prone to develop serious behavioral pathologies when housed alone (Gunn & Morton, 1995; Krohn et al., 1999; Held et al., 2001). Trichophagy is one such behavioral disorder that often results in the formation of gastric trichobezoars (Wagner et al., 1974), a clinical problem that may lead to an alarmingly high incidence of mortality associated with intestinal stasis (Jackson, 1991).

Single housing is likely to be stressful for dogs and is associated with an increased incidence of bizarre movements and barking (Hetts et al., 1992; Hubrecht et al., 1992).

In cats, single housing can result in extreme boredom manifesting in pathological behaviors such as psychogenic alopecia and polyphagia with resultant obesity (Buffington, 1991; DeLuca & Kranda, 1992).

When sheep are kept alone, they are prone to developing stereotypical pulling and chewing of wool and gnawing at wooden structures of their enclosure. "The nature and extent of the abnormal behaviour found may be indicative of a differing background physiological state"

(Marsden & Wood-Gush, 1986, p 159) which can make the extrapolation of experimental results rather problematic (Done-Currie et al., 1984). Single-housed sheep show an increase in heart, respiration and metabolic rates (Baldock & Sibly, 1990; Van Adrichem, 1993; Cockram et al. 1994; Carbajal & Orihuela, 2001; McLean & Swanson, 2004), plus an increase in adrenal and thyroid activity (Bobek et al., 1986; Bowers et al., 1993).

In goats, plasma norephinephrine levels increase when they are kept without contact with other goats (Carbonaro et al., 1992).

The deleterious effect of social deprivation is particularly pronounced in nonhuman primates. It is, therefore, a legislative imperative in the United States that:

> *Research facilities must develop, document, and follow an appropriate plan for environmental enhancement. … The plan must include specific provisions to address the social needs of nonhuman primates* (United States Department of Agriculture, 2002, p 94).

European regulations also make it clear that: "Because the common laboratory non-human primates are social animals, they should be housed with one or more compatible conspecifics. … Single housing should only occur if there is justification on veterinary or welfare grounds. Single housing on experimental grounds should be determined in consultation with the animal technician and with the competent person charged with advisory duties in relation to the well-being of the animals (Council of Europe, 2006, p 44 & 14). In rhesus macaques, the species most commonly found in laboratories, single caging can produce "long-term features of immunosuppression and significant increases in plasma prolactin concentrations, indicative of stress-induced anxiety" (Lilly et al., 1999, p 197). A major problem among individually caged primates is apathy and depression (Erwin & Deni, 1979). Clinical records and immunological data indicate that single-caged primates are more susceptible to health problems than animals living in compatible social settings (Shively et al., 1989; Schapiro & Bushong, 1994; Schapiro et al., 1997). Behavioral assessments of individually caged rhesus macaques of a Primate Research Center revealed that "of the 362 animals surveyed, 321 [88.7%] exhibited at least one abnormal behavior" (Lutz et al., 2003, p 1). Many single-caged individuals spend more than 20% of the day engrossed in abnormal behaviors (Bayne et al., 1991; Bayne et al., 1992; Kessel & Brent, 1996; Bellanca et al., 1999; Bourgeois & Brent, 2003). A particularly alarming abnormal behavior is self-injurious biting. "Monkeys with SIB [self-injurious behavior] bite their own bodies frequently, occasionally inflicting wounds" (Novak et al., 1998, p 213) that can be life-threatening (Author's unpublished observations). "Research has shown that approximately 10% of captive, individually housed monkeys have had some veterinary record of self-injurious behavior within their life-time" (Jorgensen et al., 1998, p 187). In humans, self-injurious behavior is classified as a major psychotic disorder that occurs not only in mentally handicapped individuals (Simeon et al., 1992) but also in socially isolated prisoners (Yaroshevsky, 1975). In large colonies of single-caged rhesus macaques, the incidence of this behavioral pathology may be as high as 14% (Novak, 2003) or even 39% (Alexander & Fontenot, 2003).

It is not necessary to keep animals in barren living quarters in research facilities. The provision of species-appropriate stimuli (environmental enrichment) "may reduce variability between animals and produce animals that are better models of normal function" (Garner, 2002, p 95).

Inanimate enrichment does not cure animals of behavioral pathologies but it temporarily reduces the frequency of their occurrence during the time the animals are actively engaged with the enrichment. The distraction derived from the availability of the enrichment is likely to ameliorate the overall stress attendant to boredom. Rats kept in enriched cages show longer durations of sleep and lower levels of agonistic behavior (Abou-Ismail et al., 2010), they are less timid and less afraid (Denenberg & Morton, 1962; Klein et al., 1994; Eskola & Kaliste-Korhonen, 1998) and show significantly lower baseline adrenocorticotropic hormone and corticosterone concentrations compared to rats housed without enrichment (Belz et al., 2003). Mice kept in enriched versus barren standard cages are less emotional/fearful/excitable (Manosevitz & Joel, 1973; Chamove, 1989; Scharmann, 1994; Roy et al., 2001; Van de Weerd et al. 2002; Benaroya-Milshtein et al., 2004; Õkva et al., 2010). They show lower plasma corticosterone levels (Hennesy & Foy, 1987; Roy et al., 2001) and a less variable, better regulated immune response (Kingston & Hoffman-Goetz, 1996), and are less aggressive among each other (Ambrose & Morton, 2000). Access to appropriate inanimate enrichment can diminish aggressive interactions among hamsters (Arnold & Westbrook, 1997/1998), rabbits (Mis & Warren, 2003), chickens (Yasutomi & Adachi, 1987; Gvaryahu et al., 1994; Baroli et al., 1997), pigs (Schaefer et al., 1990; Blackshaw et al., 1997; Durrell et al., 1997; O'Connell & Bettie, 1999) and primates (Neveu, 1994; Nakamichi & Asanuma, 1998; Honess & Marin, 2006). In chickens, environmental enrichment also reduces the incidence of cannibalism (Yasutomi & Adachi, 1987). In mice (Engellenner et al., 1982; Van de Weerd et al., 2002), rats (Deacon, 2001; Morrison, 2001), hamsters (Arnold & Gillaspy, 1994), rabbits (Mis & Warren, 2003), pigs (Grandin, 1986; Pearce et al., 1989; Moore et al., 1994; Rodarte et al., 2004) and chickens (Brake, 1987; Reed et al., 1993) proper enrichment buffers the animals' fear and aggressive defense response to personnel, thereby diminishing stress reactions to being captured for procedures.

Social enrichment in the form of compatible conspecific companionship avoids physiological, stress-related imbalances in single-caged gregarious animals; this has been demonstrated in mice (Goldsmith et al., 1976), rats (Ader & Friedman, 1964; Gardiner & Bennet, 1978; Fagin et al., 1983; Cambardella et al., 1994; Baldwin et al., 1995; Nyska et al., 1998), squirrel monkeys (Gonzalez et al., 1982), baboons (Coelho et al., 1991), rhesus macaques (Schaprio et al., 2000a; Doyle et al., 2008) and chimpanzees (Reimers et al., 2007). Social companionship also attenuates behavioral disorders. It actually prevents the development of stereotypies and trichophagy in rabbits (Poderscek et al., 1991; Love, 1994; Krohn et al., 1999; Held et al., 2001) and ameliorates or eliminates the behavioral pathology of self-biting (Fritz, 1989; Line et al., 1990; Reinhardt, 1999; Weed et al., 2003) as well as self-directed hair pulling (Hartner et al., 2000; Reinhardt, 2010) in chimpanzees and macaques.

Optional visual seclusion is an essential environmental enrichment/refinement, fostering the well-being and physiological equilibrium of confined animals. Individually housed animals may seek seclusion for undisturbed resting, while socially housed animals may seek cover to diffuse social tension and avoid aggressive conflicts. Access to a covered retreat area is particularly important during alarming situations—for example, when fear-inducing personnel enter the room. Furnishing the tanks of frogs with hollow structures for hiding significantly decreases both aggression and mortality rate (Hedge et al., 2002; Torreilles & Green, 2007); it also diminishes startle responses, suggesting that the frogs get less stressed when personnel are around (Archard, 2012). In groups of male mice, access to soft paper material decreases fecal corticosterone levels and reduces aggressive interactions by offering subordinates cover and escape routes (Armstrong et al., 1998; Van Loo et al., 2002; Niu et al., 2011). Pair-housed male guinea pigs show significantly lower fecal cortisol concentration when they are provided with a hut serving as a buffer against social tension (Walters et al., 2012). Common rats with access to shelters are less timid and engage in stereotyped backflipping less often than those in barren cages (Townsend, 1997; Callard et al., 2000). Cotton rats become less aggressive among each other and against personnel when they are provisioned with tubes serving them as refuge places (Neubauer & Buckmaster, 2011). Hamsters often develop bizarre aggressive behaviors when housed individually in suspended wire cages. Providing a piece of polyvinyl chloride pipe as a place for seclusion can resolve this problem (McClure & Thomson, 1992). Aggression is reduced among rabbits when sections of their pens are screened, tubes are placed on the floor or shelves installed so that the animals can withdraw or escape from each other as needed (Howard et al., 1999; Stauffacher, 2000). Visual barriers reduce aggression among pigs (Waran & Broom, 1993), cattle (Bouissou, 1970) and primates (Erwin, 1977; Reinhardt & Reinhardt, 1991; Maninger et al., 1998; McCormack & Megna, 2001). Placement of vertical panels in the middle of the enclosure assures that chickens—and presumably also rodents—show less disturbance reactions and make use of the available floor space more evenly by no longer aggregating at the peripheral walls and shunning the otherwise unprotected central area (Newberry & Shackleton, 1997; Cornetto & Estevez, 2001; Cornetto et al., 2002).

Music and/or talk radio: Attending care personnel often listen to music or talk radio while doing their routine work in the animal rooms. The type of music and the type of talk radio listened to will differ from person to person, and there will be some caregivers who do their work in silence.

Music can have a calming effect in dogs, as exposure to new age music decreases the amount and intensity of their barking (Kilcullen-Steiner & Mitchell, 2001). Radio music can make laying hens more productive (Jones & Rayner, 1999), suggesting that music affects their endocrine system.

Exposure to gentle music has a calming effect on mice (Chikahisa et al., 2007; Li et al., 2010) and boost their immune system (Núñez et al., 2002).

Rats are able to distinguish between different radio sound patterns; they show a clear preference for silence to anything else, which may be taken as an indication that they feel disturbed by the sound from the radio (Krohn et al., 2011). Hearing music modifies their cardiovascular functions by either increasing or decreasing heart rate and blood pressure, depending on the tempo, rhythm, pitch and tonality of the music (Lemmer, 2008; Akiyama & Sutoo, 2011).

Low-volume music is likely to reduce the intensity of the startle response that rabbits typically exhibit when fear-inducing personnel enter their room (Reinhardt, 2010). Music can have a very calming effect on pigs; they will quietly lie in body contact with each other and barely move when personnel enter their room (Reinhardt, 2013).

In primates, certain types of music have no noticeable effect (e.g., harp music, vervets: Hinds et al., 2007), or have a calming effect as reflected in lowered heart rate and increased resting behavior (e.g., oldies, baboons and chimpanzees: Brent & Weaver, 1996; Howell et al., 2003), while other types of music can make the animals more restless (e.g., high-beat, chimpanzees: Harvey et al., 2000). A study with tamarins and marmosets showed that the animals prefer slow-tempo to fast-tempo music, but when allowed to choose between slow-tempo music and silence, they prefer silence (McDermott & Hauser, 2007). Salivary cortisol concentrations doubled in marmosets following 30 minutes of exposure to radio music/talk (Pines et al., 2004), suggesting that the radio is a source of stress rather than entertainment for these animals.

Music and/or talk radio may be a pleasant distraction for attending animal care personnel (Reinhardt, 2010) but a stressful disturbance for the confined animals, especially those who are nocturnal (e.g., mice and rats) and want to sleep during the day. Before animals are exposed to regular noise from radios, a simple preference test should be conducted to find out if the animals actually prefer the noise over silence. If they prefer silence, no music and/or radio talk should be played in their holding areas, not only for the sake of animal well-being but also for the sake of sound scientific methodology.

Removal from the home environment

Animals in research institutions are regularly caught and transferred to treatment, test or experimental areas for procedures that entail involuntary restraint. The stress/distress resulting from enforced restraint is augmented by the stress resulting from the transfer to an unfamiliar treatment area. For example, in rats and mice, even moving animals in their familiar cages to an unfamiliar room in the same building increases corticosteroid levels for several hours (Kvetnansky et al., 1978; Ursin & Murison, 1986; Drozdowicz et al., 1990; Tuli et al., 1995a). "Exposure to a new environment, or novelty, may contribute significantly to the adrenocortical response often attributed to the effects of noxious or painful stimulation" (Friedman & Ader, 1967). Dobrakovavá & Jurcovicova (1984) tried to adapt male Wistar rats, caged in groups of four, to being transferred in their home cage to another room, stay there for 10 minutes, and then return to the original location. This procedure was repeated once a day for a 15-day period. The animals showed a significant increase in plasma corticosterone and prolactin concentration in response to the transfer procedure on day 15, to levels that were not lower than those observed on day 1.

> *Wherever possible, every effort should be made to design in ways that bring the treatment to the animal, instead of the reverse. Removal for any purpose exposes the animal to overly novel, frequently noxious, and always stressful stimuli* (Lindburg & Coe, 1995, p 565).

Novelty of the environment activates the pituitary-adrenal axis not only in rodents but also in nonhuman primates (Friedman & Ader, 1967; Brown & Martin, 1974; Pfister & King, 1976; Line et al., 1987; Cabib et al., 1990). "Removing an animal from its home cage prior to monitoring anything biological will probably affect the event being monitored" (Mitchell & Gomber, 1976, p 546). Single-caged rhesus macaques show an increase in self-biting behavior, evidence of sleep disturbance, and elevated cortisol levels in saliva and serum after being removed from their familiar living quarters to unfamiliar living quarters (Davenport et al., 2008). The magnitude of cortisol response to blood collection is significant when the procedure occurs in the hallways but not when it occurs in the home cage (Herndon et al., 1984; Reinhardt et al., 1990; Reinhardt et al., 1991). Macaques show significantly higher cortisol and catecholamine levels when they are chair-restrained in an unfamiliar versus a familiar environment (Mason 1972; Mason et al., 1973).

Investigators often fail to mention in scientific articles if their research subjects were removed or if they were allowed to stay in the familiar home environment during procedures (Reinhardt & Reinhardt, 2000b). To ignore this variable while ascertaining *basal* or *normal* values of stress-sensitive parameters would contravene basic scientific rules.

Removal from social companion(s)

Experiments and tests are often a source of stress or distress; in addition, they usually entail the subject being removed not only from the familiar home environment but also from familiar conspecifics. Any gregarious animal who is unwillingly removed from familiar social companions experiences separation stress/anxiety/depression, accompanied by significant physiological and biochemical reactions that are bound to influence subsequently collected physiological data (*rats*: Ehlers et al., 1993; *mice*: Pibiri et al., 2008; *pigs*: Ruis et al., 1997; *sheep*: Apple et al., 1993; *cattle*: Hopster & Blokhuis, 1993; *chickens*: Jones & Merry, 1988; *primates*: Pearson et al., 2008).

There is ample evidence indicating that the presence of one or several companions has a stress-buffering effect, reducing both the magnitude and frequency of physiological stress reactions to aversive circumstances. When rodents are exposed to a stressful situation, physiological stress reactivity is buffered by the presence of another conspecific (*rats*: Davitz & Mason, 1955; Conger, 1957; Latané & Glass 1968; Taylor 1981; Giralt & Armario, 1989; Heath, 1999; de Jong et al., 2005; *mice*: Goldsmith et al., 1978; Van Loo et al., 2007; *guinea pigs*: Sachser et al., 1998; Kaiser et al., 2003; Hennessy et al., 2006; *hamsters*: Detillion et al., 2004).

In ruminants, the stress associated with experimental conditions is buffered by a familiar conspecific (*sheep*: Pearson & Mellor, 1976; Baldock & Sibly, 1990; Fraser, 1995; *cattle*: Veissier & Le Neindre, 1992).

In nonhuman primates, the presence of a companion reduces signs of behavioral disturbance and the magnitude of cortisol increase during a fear-provoking situation (Vogt et al., 1981; Coe et al., 1982; Gonzalez et al., 1982; Stanton et al., 1985). Individuals recover from the stress of being transferred to a novel environment significantly faster when a companion is present than when they are alone (Gust et al., 1994). Being tethered during an experiment is an extremely disturbing situation (Kaplan et al., 1983; Adams et al., 1988; Crockett et al., 1993). The cardiovascular stress response is significantly lower when tethered subjects are allowed to keep visual, tactile and auditory contact with other conspecifics than when they are kept alone (Coelho et al., 1991). "Social stimuli may function as a source of security and a means of mitigating emotional distress" (Mason, 1960, p 110).

For common husbandry- and research-related procedures there is often no need to remove animals from their familiar living quarters and separate them from social companions. This applies particularly to nonhuman primates.

It has been documented that socially housed marmosets readily learn to (a) step, one at a time, on a scale and remain still during weighing (McKinley et al., 2003) and (b) cooperate during urine sample collection (Anzenberger & Gossweiler, 1993; McKinley et al., 2003). Group-housed tamarins, vervet monkeys, and chimpanzees can also be trained to reliably produce urine samples in their home enclosure (Kelley & Bramblett, 1981; Snowdon et al., 1985; Stone et al., 1994; Lambeth et al., 2000). Chimpanzees living in a social setting can be trained to provide semen samples, present for subcutaneous and intramuscular injection, and cooperate during blood collection without needing to be removed from companions (Perlman et al., 2003; Schapiro et al., 2005; Coleman et al., 2008). Group-housed stump-tailed macaques can readily learn to present for vaginal swabbing within their home enclosure (Bunyak et al., 1982). Pair-housed stump-tailed macaques learn easily to cooperate during topical drug application in their home cages (Reinhardt & Cowley, 1990). Pair-housed stump-tailed and pair-housed rhesus macaques have been trained to voluntarily present for blood collection in their familiar living quarters (Reinhardt et al., 2002). It has been shown in pair- and group-housed marmosets and baboons that saliva samples can be obtained without difficulties from individual animals without having to remove them from their group (Cross et al., 2004; Pearson et al., 2008).

Group-housed rats learn quickly to cooperate during oral drug delivery in their home cages (Huang-Brown & Guhad, 2002; Rourke & Pemberton, 2007).

Multi-tier caging

Small and medium-size animals are traditionally kept in rows of cages that are stacked on top of each other so that a maximum number of animals can be accommodated per room. This creates different living environments in the cages of different racks in terms of:

1. distance from the light source and
2. distance from the floor of the room.

A bright upper-row cage may provide a species-suitable environment for a diurnal arboreal animal such as a primate, but it would be unsuitable for a nocturnal terrestrial animal such as a rodent (Ader et al., 1991). Yet, many of the caged primates live in the crepuscular environment of lower rows while many of the caged rodents live in the bright environment of upper rows.

It should be noted here that US animal welfare regulations pertaining to dogs, cats, guinea pigs, hamsters, rabbits and primates explicitly stipulate that:

> *Lighting must be uniformly diffused throughout animal facilities* (United States Department of Agriculture, 2002, pp 58, 72, 80, 90).

This unequivocal legal requirement is a safeguard for reliable scientific methodology, as light influences physiological systems, metabolism, general activity, behavior and emotionality (Marshall, 1940; Ross et al., 1966; Wurtman, 1967; Weihe et al., 1969; Martinez, 1972; Hauntzinger & Piacsek, 1973; Vriend & Lauber, 1973; Weihe, 1976; Newton, 1978; Saltarelli & Coppola, 1979; Clough, 1984; Heger et al., 1986; Martin, 1991). "The illumination conditions existing around the animals in their cages need to be considered for each species and for a much wider range of functions than has previously been thought necessary" (Weihe, 1976, p 74). A difference in light quantity and light quality between locations on a cage rack constitutes an extraneous variable that has to be taken into account if the validity of scientific research methodology is to be warranted (Ott, 1974; Canadian Council on Animal Care, 1993). Surprisingly, it is rarely mentioned in scientific articles whether the animals of the research project were *all* caged at the *same* level of the room (cf., Davis et al., 1973; Claassen, 1994).

With multi-tier caging it is difficult to provide uniform lighting for all animals since upper rows will always cast shade on cages in lower rows. Not only the quantity but also the quality of light in lower rows differs from that in upper rows. The light that lower-row-caged animals are receiving is not direct but is reflected from the walls of the room, thereby changing its spectral distribution, depending on the colors of the walls. It is probably not an overstatement that:

> *The intensity of light in animal cages is likely to be the most variable environmental factor in the average animal room* (Clough, 1982, p 512).

"What we basically have done to date is to provide lighting suitable to our needs and assumed it was all right for the animal" (Bellhorn, 1980, p 441)—and for research. In standard multi-tier rodent cages and in standard double-tier primate cages, variation in light intensities far exceeds a two-fold difference between bottom- and top-row cages (Clough & Donnelly, 1984; Reinhardt & Reinhardt, 1999; Schapiro et al., 2000b).

The International Primatological Society (2007, p 12) notes that "A two-tiered system is not recommended as these cages are usually too small. The lower tiers do not allow primates to engage in their vertical flight response, are often darker, and animals in the lower cages tend to receive less attention from attending personnel." The bottom-row cages are often so dark that it is difficult to identify and check individual animals without the use of flashlights (Reinhardt, 1997; Reasinger & Rogers, 2001; Savane, 2008). This situation not only introduces an uncontrolled variable into research data but also undermines good housekeeping. Surprisingly, it is mentioned in primatological research articles only rarely—2% of 96 articles surveyed—whether the research subjects were housed at different levels or if all of them were housed at the same level of the multi-tier cage rack (Reinhardt & Reinhardt, 2000b).

Multi-tier caging systems also make it impossible to provide all animals of a room the same feeling of security when personnel approach them. Especially the investigator constitutes a potential stressor, often triggering pronounced physiological and behavioral disturbance in caged subjects (*mice*: Kramer et al., 2001; *rats*: Döhler et al., 1977; *monkeys*: Tatoyan & Cherkovich, 1972; Malinow et al., 1974; Manuck et al., 1983; Hassler et al., 1989; Line et al., 1989; Schnell & Wood, 1993; Bowers et al., 1998; Boinski et al., 1999; Crockett & Gough, 2002), presumably due to aversive conditioning (Robbins et al., 1986). Empirical evidence indicates that the fear response to personnel differs in animals kept in upper rows versus those kept in lower rows. In chickens, fear reactions are moderate when they are kept in bottom-row cages but intense when they are kept in upper-row cages (Sefton, 1976; Hemsworth et al., 1993). "Under natural conditions, many primates spend much of their lives above ground and escape upward to avoid terrestrial threats. Therefore, these animals might perceive the presence of humans above them as particularly threatening" (National Research Council, 1998, p 118). A monkey who is kept in a bottom-row cage is practically cornered when being approached by a person, while a monkey in an upper-row cage can retreat to a relatively safe place above the person. The different emotional reactions of upper-row versus lower-row caged animals are likely to impact differently the subjects' physiological responses during subsequent experiments.

Rotating cages through different positions on a rack (Ross & Everitt, 1988; Canadian Council on Animal Care, 1993; National Research Council, 1996) rotates the methodological problems arising from the multi-tier caging but it does not solve them. Cage-specific light sources could avoid differences in illumination (MacLean et al., 2009; Baumans et al., 2013) but not differences in cage distance from the floor of the room. Presently, there is no other alternative than single-tier caging that can provide all animals of a room a uniform housing environment, both in terms of distance from the light source and distance from the floor.

Restraint

It is common practice in research laboratories to restrain animals during drug administration and sample collection procedures (Wolfensohn & Lloyd, 1994; Fowler, 1995; Hrapkiewicz et al., 1998). Being caught and subsequently immobilized by a predator—such as a human—is a life-threatening, hence distressing experience for any animal, purpose-bred beagles probably being the exception.

> *Because restraint itself is a stressor that affects the physiological functioning of the subject, measurement error and variability are introduced into the data* (Brockway et al., 1993, p 57).

"Since the purpose of physiological recording should be to obtain a record that is an exact facsimile or analog of the events under investigation, stress induced by restraint and handling, even when these are of minor nature and performed by skilled staff, is one of the major problems encountered in biomedical investigations" (Schnell & Gerber, 1997, p 68). "It is only common sense ... that an animal will not respond normally if it is stressed" (Schwindaman, 1991, p 30).

Manual restraint is usually applied with rats and mice. The animals show a variety of stress reactions that may include:

- » increase in adrenocortical activity (Tuli et al., 1995b),
- » activation of adrenal-medullary discharge of epinephrine and a sympathetic neuronal release of norepinephrine (Kvetnansky et al., 1978),
- » increase in heart rate (Harkin et al., 2002; Sharp et al., 2003),
- » changes in plasma prolactin levels (Krulich et al., 1974; Lenox et al., 1980; Gala & Haisenleder, 1986),
- » increase in plasma glucose level (Besch & Chou, 1971),
- » increase in core temperature (Berkey et al., 1990; Harkin et al., 2002), and
- » alterations of metabolism and excretion of drugs (Kissinger et al., 2001).

Even witnessing the restraint of another conspecific can be enough to trigger stress reactions in unrestrained animals (Pitman et al., 1988; Fuchs et al., 1987; Sharp et al., 2002b).

For hamsters, routine manual restraint is a stronger stressor—as measured in heart rate increase and core body temperature increase—than intruder confrontation, cage change, and group formation (Gattermann & Weinandy, 1996/97). Guinea pigs often exhibit signs of fear (struggling) and anxiety (squealing) when they are manually restrained for procedures (Author's unpublished observations). This suggests that they also experience stress-triggered physiological alterations when being restrained.

In sheep, restraint increases cortisol, lactate, and glutamic oxaloacetic transaminase (Apple et al., 1993).

In pigs, enforced restraint raises cortisol levels, induces prostaglandin-mediated hyperthermia, and affects the acid-base balance (Van de Wal et al., 1986; Parrott & Lloyd, 1995).

In ferrets, manual restraint results in a significant increase of plasma cortisol and adrenocorticotrophic hormone and a decrease of alpha-MSH (Schoemaker et al., 2003).

In a study with dogs, an acclimatization period of at least 4 weeks was required to notably reduce stress-related effects associated with periodic manual restraint during venipuncture. Epinephrine (adrenaline) values gradually declined, but they were still almost 50% above baseline by the end of the study on day 41 (Slaughter et al., 2002).

Cats do not like to be restrained and will take every opportunity to get free, even if this implies defensive aggression against the handling person (Author's unpublished observations). A cat's aversive reactions suggest that enforced restraint is a stressor that is likely to affect the animal's physiological equilibrium.

Involuntary restraint is an especially distressing experience for nonhuman primates. Subjects are restrained either manually or mechanically during brief sample collection or drug administration procedures; they are strapped into restraint chairs or on restraint crosses during long-term procedures (Fowler, 1995). Restrained individuals usually struggle, exhibit self-defensive aggression, and often squeal. Physiological reactions to brief restraint include:

- increased respiration rate (Berendt & Williams, 1971),
- metabolic acidosis (Manning et al., 1969),
- increased heart rate (Osborne 1973; Line et al., 1991; Schnell & Wood, 1993),
- increased blood pressure (Golub & Anderson, 1986; Schnell & Wood, 1993),
- raised rectal temperature (Bush et al., 1977),
- rise in SGO-T (serum glutamic-oxalacetic transaminase) (Cope & Polis, 1959),
- rise in AST (aspartate aminotransferase) and ALT (alanine aminotransferase) (Landi et al., 1990),
- increased plasma cortisol concentrations (Elvidge et al., 1976; Puri et al., 1981; Fuller et al.,1984; Suzuki et al., 2002),
- leukocytosis (Ives & Dack, 1956; Loomis et al., 1980; Goosen et al., 1984),
- increased plasma concentrations of adrenal androgens (Fuller et al., 1984),
- elevation of plasma prolactin (Quadri et al., 1978),
- increased glucagon levels (Myers et al., 1988),
- impaired glucose clearance (Yasuda et al., 1988),
- impaired testosterone release (Puri et al., 1981; Hayashi & Moberg, 1987; Torii et al., 1993),
- baseline variability in growth hormone levels (Mason et al., 1968), and
- alterations of the electrocorticogram (Bouyer et al., 1978).

Even after repeated (12 times) exposure to brief restraint in the familiar home cage, rhesus macaques continue to show a pronounced heart-rate response, indicating that they do not habituate to this common procedure (Line et al., 1991).

The sedative ketamine is often injected with the assumption that it will reduce the overall stress that primates experience during the most common procedure, namely blood collection (Laudenslager & Worlein, 2003). Traditionally, the subject is forcibly restrained during the injection, a circumstance that introduces restraint-stress as an extraneous variable even before the blood has been drawn (Aidara et al., 1981; Bentson et al., 2003). Ketamine sedates the animal for the subsequent blood collection but it does not modify the cortisol response to the initial enforced injection of the ketamine (Loomis et al., 1980; Puri et al., 1981; Fuller et al.1984; Crockett et al., 2000). It is questionable that reliable control values of stress-sensitive blood parameters can be obtained under such conditions. The statement that "simple procedures such as injections of relatively harmless substances and blood sampling ... are expected to produce little or no discomfort" (Scientists Center for Animal Welfare, 1987, p 12) is valid only under the condition that the subject is *not* forcibly restrained during these procedures.

> [Possibly data-influencing] *physiological, biochemical and hormonal changes occur in any restrained animal. ... Restraint procedures should [therefore] only be invoked after all other less stressful procedures have been rejected as alternatives* (Canadian Council on Animal Care, 1993, p 95).

Alternative "procedures that reduce reliance on forced restraint ... are less stressful for animals and staff, safer for both, and generally more efficient" (National Research Council, 1998, p 45).

Biotelemetry offers alternative means of obtaining physiological measurements from freely moving animals, without introducing stress artifacts resulting from restraint. This system consists of a radio transmitter placed in a jacket or implanted subcutaneously or intraperitoneally, depending on the species and size of the subject. It has been used successfully in mice, rats, gerbils, guinea pigs, hamsters, rabbits, dogs, cats, primates, chickens, goats, sheep, amphibians, reptiles and fishes to monitor activity, body temperature, heart rate, blood pressure, electrocardiogram, and electroencephalogram (Malinow et al., 1974; Laburn et al., 1992; Kramer et al., 1993; Schnell & Wood, 1993; Depasquale et al., 1994; Christian & Bedford, 1995; Sato et al., 1995; Truett & West, 1995; Colbourne et al., 1996; Dejardins et al., 1996; Savory & Kostal, 1996; Brown et al., 1997; Heybring et al., 1997; Seebacher & Alford, 2002; Bridger & Booth, 2003; Krohn et al., 2003; Morton et al., 2003; Van Ginneken et al., 2004).

Positive reinforcement training is an alternative to involuntary restraint during procedures that necessitate the direct handling of the subject.

> *The least distressing method of handling is to train the animal to co-operate in routine procedures. Advantage should be taken of the animal's ability to learn* (Home Office, 1989, p 18).

Training animals to cooperate rather than resist during procedures not only refines research methodology but also improves personnel safety; a cooperative animal who works *with* the handling person has no reason to resort to self-defensive aggression.

In rats and rabbits, the stress and risk associated with gastric intubation can be avoided by training the animals to accept and swallow test drugs (e.g., indomethacin, celecoxib, tosufloxacin) that are masked with chocolate or sucrose (Marr et al., 1993; Huang-Brown & Guhad, 2002). Eight of ten rabbits cooperated within 2 days. They "would stand with their paws on the front of the cages, protrude their faces from between the bars, and appear to beg for the syringe containing the antibiotic" (Marr et al., 1993, p 46). Rats have successfully been trained to cooperate during saliva collection in their familiar home environment (Guhad & Hau, 1996).

Sheep can easily be trained to voluntarily enter a tilt table and accept brief immobilization for a grain reward (Grandin, 1989). It takes pigs just 2 weeks of training before they voluntarily run down the hallway to get onto a platform scale, where they stand still and enjoy a food reward while their weights are being recorded (Neubauer et al., 2011). Göttinger minipigs learn easily to voluntarily come forward, step onto a box, and hold still during dosing in one nostril (Brodersen et al., 2010); they also learn to follow a target stick, walk onto a scale for weighing, and stand still for physical examinations, electrocardiography, and dermal dosing (Blye et al., 2006).

Primates readily learn to cooperate during injection (Spragg, 1940; Levison et al., 1964; Priest, 1991; Nelms et al., 2001; Bentson et al., 2003), blood collection (Wall et al., 1985; Hein et al., 1989; Priest, 1990; Reinhardt, 1991; Reinhardt & Cowley, 1992; Priest, 1998), saliva collection (Bettinger et al., 1998; Tiefenbacher et al., 2003), urine collection (Kelley & Bramblett, 1981; Anzenberger & Gossweiler, 1993; Schnell & Gerber, 1997; Lambeth et al., 2000; McKinley et al., 2003), vaginal swabbing (Bunyak et al., 1982), oral drug administration (Turkkan et al., 1989; Klaiber-Schuh & Welker, 1997; Schnell & Gerber, 1997; Crouthamel & Sackett, 2004; Reinhardt, 2013) and topical drug application (Reinhardt & Cowley, 1990). Trained subjects show no behavioral signs of fear or distress and the physiological stress response to the procedure is considerably reduced or eliminated altogether (Michael et al., 1974; Elvidge et al., 1976; Reinhardt & Cowley, 1992; Schnell & Gerber, 1997; Reinhardt, 2003; Bentson et al., 2003). Macaques can be trained to cooperate for pole-and-collar transfer to a restraint chair (Down et al., 2005; Bliss-Moreau et al., 2013; McMillan et al., 2014).

Conclusions

The variables summarized in this chapter are not the only ones that have the potential to increase variance and reduce the reliability of research data, but they are the most obvious ones that are often overlooked in research protocols.

It is a prerequisite of truly scientific biomedical research methodology to take extraneous variables into account and investigate *prior* to the experiment if one or several of them have the potential to confound the effects induced by the experimental manipulation. Eliminating or avoiding variables that can interfere with the research subject's response to a test situation is a safeguard that the variance of the collected data will be minimal. This, in turn, will enable investigators to assess their findings statistically with a minimum number of research subjects. It is unethical to use more animals in order to increase statistical power and achieve significance of research findings, rather than make an effort to assess husbandry-related variables and eliminate or avoid them if they alter the research subjects' responses to given experiments or tests (Öbrink & Rehbinder, 1999). At a very minimum all husbandry-, housing-, and handling-related variables should be adequately described in any scientific publication so that other investigators can repeat the experiment/test or carry out comparative studies (Morton, 1992; Smith et al., 1997).

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Human-Animal Bond

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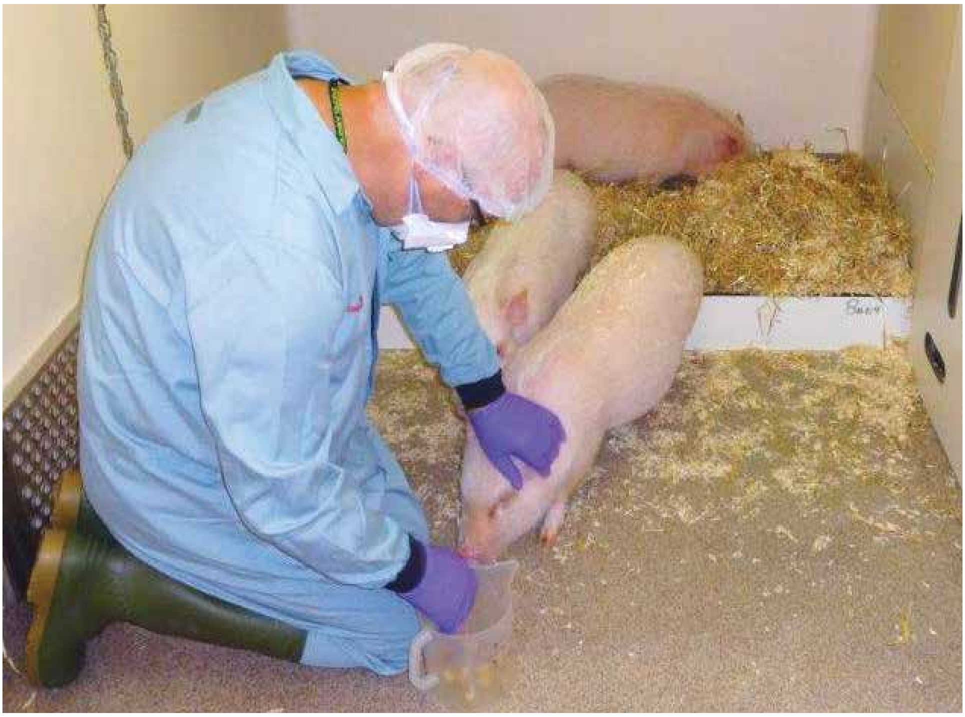
FEW WOULD QUESTION the strength of the bond that people have with their pets. Yet, to suggest a similar bond between a caretaker and a laboratory animal seems incongruous to many, even within the research setting. However, such bonds can exist and can enrich the lives of the animals and the people caring for them. Furthermore, close bonds can help science, as caretakers are able to more readily observe changes in behavior signifying potential health and welfare issues.

Over the thousands of years that humans have interacted with domesticated animals, our relationship with them has taken many different forms, including: worship, food, labor, and sport. We often use anthropomorphism when describing animals and their behavior. In some cases, anthropomorphism can be taken to the extreme, such as in the case of performing a marriage ceremony for two dogs (Podberscek et al., 2000). However, it is difficult to judge these cases, as the interpretation of the human-animal relationship varies greatly between and within societies.

When we are working with animals it is extremely important to have knowledge of the species and, in some cases, the individual animal. One may argue that what makes a great animal caretaker is someone who not only has knowledge, but also empathy with the animals in his or her charge. But how can we be sure we can empathize with animals, if we are uncertain of the causes of their behavior? Social psychologist Lauren Wispe (1987) offers the following observations:

- » The object of empathy is understanding.
- » The object of sympathy is the other [individual's] well-being.

One might argue that to be a compassionate caretaker, one must not only empathize, but sympathize with the animal.



The Farm Animal Welfare Council developed fundamental requirements for farm animals, known as the "five freedoms," which have become prominent in all industries that use animals (Farm Animal Welfare Council, 1997). These are—

» Freedom from thirst, hunger and malnutrition;
» Freedom from discomfort;
» Freedom from pain, injury and disease;
» Freedom to express normal behavior; and
» Freedom from fear and distress.

The following question was raised on the Laboratory Animal Refinement & Enrichment Forum (LAREF) (Anonymous, 2003): "Should animal care personnel be encouraged to establish affectionate, rather than neutral, relationships with the animals in their charge?" Most forum participants who responded to the question felt that the development of an affectionate relationship with the animals in their charge was almost unavoidable. The consensus was that emotional attachment provides an assurance that the animals would receive optimal care, both physically and behaviorally. As one respondent observed,

> *Having a close relationship with your animals is necessary to regard them as living beings, rather than biological test tubes. As such, you are more careful and patient, and will think more about what the procedures mean to the animals. You will become more creative in finding animal friendly alternatives for the procedures you need to do on the animals. You will thus increase the well-being of your animals and, by doing so, make them better research subjects and increase the validity of the test results.*

One individual, however, expressed concern that establishing an affectionate relationship with experimental animals and knowing them as individuals might hamper her impartiality and capacity to be objective when observing and registering their behavior. Another person countered:

> *It seems to me that we get hung up on trying to divorce our emotions from what we hope to be our objectivity. I do not think that any normally functioning human being in the world does anything for any reason other than emotional. Sure, research is done to answer questions, but isn't the premise of all research to make human (or animal) lives better? If you want to make lives better, it's because of emotion, not because you are logically attached to life.*

While there was disagreement about whether it was more difficult to establish a relationship with some animal species, it was postulated that working closely with individual animals or small groups and observing them for extended periods of time was a more important bonding factor than evolutionary relatedness with our own species. Such close contact provides insight into the personalities of individual animals and allows the caretaker to give more personalized care. An example of this was described by a colleague (personal communication, August 21, 2014):

> *I spent 6 fantastic years working within a Small Primate Unit and during this time it was amazing to see how staff and marmosets built relationships with each other. Each marmoset had a unique personality, which had to be taken into account during the daily routines. Some animals would gradually build a greater trust and bond with individual members of staff and this would not always be replicated with other members of the team. For example, Happy initially was quite a timid animal, though over time built a close bond with me. He would sit on my shoulder and was content to be escorted around the unit—we had an understanding that he was safe on my shoulder and he should not leave there unless told to do so. However, even after several years he still showed some distrust to most other members of staff.*

Barriers to human-animal bonding in the research setting

Within the research setting there are many barriers to forming a meaningful human-animal bond. This section discusses some of the most prominent.

For many animals in the research setting, their only interactions with human beings are when they are handled. Subtle differences in how the animals are handled can have significant effects on how they view human beings, in turn affecting the opportunity for a bond to form. For example, the traditional way laboratory mice are handled (i.e., picking up by the scruff of the neck or the base of the tail) is not conducive to providing the animals with a sense of security—the first step towards bonding with their caretaker (Baumann et al., 2007). Hurst & West (2013) found that using a handling tube or gently cupping mice reduced their anxiety in behavioral studies. Hurst suggested that picking mice up by the tail was similar to how a predator would catch the mice and thus, to reduce their fear this traditional method should be avoided.

Bonding with mice may seem unrealistic due to the vast number of these animals typically housed in a facility. However, if caretakers took just 1 minute to really observe an occupied mouse cage, they would notice that each mouse has a unique personality and is reacting differently to the observer's presence (Baumann et al., 2007). Seeing each mouse as an individual improves the likelihood of forming a sympathetic bond with them.

Other significant barriers to the formation of bonds are the cages (particularly for rodent species) and personal protective equipment (PPE) requirements (particularly for nonhuman primates). This barrier is particularly prominent with the use of Individually Ventilated Cages (IVCs). Among the reasons IVCs are used:

- They foster a cleaner working environment.
- They remove most of the odors associated with rodents.
- There is less disturbance to the animals when people enter the housing room.
- They can effectively isolate health issues.
- They minimize human exposure to rodent allergens.
- They provide very efficient use of space, allowing far greater numbers of animals to be housed in a single room when compared to conventional housing options.

Unfortunately, these otherwise positive aspects create an almost unbreakable barrier to forming any bond with the cage inhabitants. Caretakers are encouraged to minimize contact with the animals, often only looking at them briefly each day, through the cage side. In larger facilities, the time pressures of changing several hundred cages daily prevent more than cursory contact with the animals. This production-line mentality can lead to an emotional detachment from the animals, creating a negative or disinterested view of them. When the focus shifts to volume-driven factors, the first things that tend to be lost are personal touches that improve animal welfare. Consider the contrast to what this caretaker describes (personal communication, September 8, 2014):

> *When I started working with laboratory animals we could walk from room to room and when I had spare time at the end of the day I could wander round the facility and handle the animals. I used to make a bee line for the rats. I used to just sit there with a whole cage-worth either in my pockets or on my lap exploring because in those days we had time to devote to animal playtime.*

If it is difficult or impossible for us to establish positive relationships with the animals in our care, we may become disengaged from their well-being. "Researchers must continue to question the barriers that have traditionally been erected against forming HABs [human-animal bonds] in the name of objectivity and to investigate seriously the ways in which fostering the formation of HABs can promote animal welfare without compromising the scientific respectability of research" (Russow, 2002).

In 2002, the Institute of Animal Technology commissioned a survey of all its 2,000-plus members, asking a number of questions regarding their professional motivations; the Institute received 511 responses (Institute of Animal Technology, 2002). Some of the reasons people choose to work with laboratory animals include the following:

- They are fascinated by working with the animals, and enjoy observing them.
- They enjoy handling and interacting with the animals in their charge.
- They like attending to the animals' needs and improving their quality of life.

Addressing these reasons can be very difficult within a modern research facility, where rodents are primarily housed in IVCs. The remainder of the chapter will discuss ways to promote the human-animal bond, focusing on the most commonly used animals in research.

Building a trust relationship with animals

Any relationship between a human and an animal is based on mutual trust that the relationship will not result in harm. Without that trust, a bond can never develop. For example, in describing his behavioral work with bison, Reinhardt (personal communication, August 2014) explained:

> *When studying the behavior of bison, I did bond with several animals. You can imagine that getting close to a one-ton bison bull implied that the bull had learned that he could trust that I was not going to harm him. Concurrently, I learned that I could trust that he wouldn't harm me. So there was no reason to be afraid; we both felt safe.*

As noted above, handling is the primary interaction between animals and their caretakers. Proper handling is perhaps the most important step to building the trust relationship required to form an actual bond. For example, even minimal, gentle handling can help rats become very friendly towards handlers. They appear to perceive human interactions as a positive experience. This was demonstrated in a study by Cloutier et al. (2014) where one handler tickled rats for 2 minutes a day, whereas a second handler restrained them. When the animals were placed in an environment with access to both handlers' hands they tended to interact more with the handler who had tickled them, engaging in gentle nibbling of the hand, which was interpreted as a friendly, playful behavior. Davis & Perusse (1988) showed that rats will actually work to be petted by a preferred human, even in the absence of a reward such as food. Studies like these demonstrate how easily the animals will form a bond with people, when given a positive environment.

Turning the focus to another research animal: laboratory rabbits maintain many traits of their wild counterparts and can be very nervous around people. (Mykytowycz & Hesterman, 1975). Several studies have shown that gentle, compassionate handling helps rabbits to overcome their fear of humans and makes them more compliant during handling procedures (Podberscek et al., 1991; Swennes et al., 2011). Spending a few minutes a day talking to rabbits, encouraging them

to come forward, and stroking them, decreases their fear of humans. Over time, rabbits can even get quite attached to caregivers and may hop up to the front of the cage for attention.

Again, spending time with the animals allows the caregiver time to discover the animals' distinct personalities and preferences. The following anecdote comes from another caregiver (personal communication, September 8, 2014):

> *Several years ago I worked with 3 NZW* [New Zealand White] *rabbits; I called them each a name that I thought related to their personality to help me remember any individual rabbit's quirks. Gemma used to grunt when I took her hay away to give fresh. It's nothing special but* [giving her a "G" name] *helped me remember what was her quirk and not abnormal for her. Madison (Mad) always tried to bite me when I opened the cage, so one day I just opened the cage* [and] *laid my arms across the floor. I wasn't sure what he would do to be honest; I just stayed there to see if he would come forward. He slowly did and, to my surprise, started rubbing his chin on my arms. This continued and he eventually trusted me to remove his hay and give him fresh hay without trying to bite me. He wouldn't let me stroke him. I think this was just his preference because as soon as I opened the cage he would run forward for me to put my arms down so he could scent mark me! I felt like we both reached an understanding. On the other hand, Rodger loved being stroked. As soon as you went near the cage he would run to the front and lay his head down for you to stroke him.*

Consistent handling of rabbits not only decreases their fear of humans but it will also make experimental results more reliable (Verwer et al., 2009) by avoiding physiological stress reactions.

Establishing mutual trust, while bonding with animals, provides the foundation for successful cooperation rather than resistance during the daily husbandry and research procedures. It has been documented in the literature that virtually all animals in research, including nonhuman primates (Turkkan, 1990), rats (Rourke & Pemberton, 2007), rabbits (Marr et al., 1993), dogs (Roddis, 2005), cats (Albertin, 1990), pigs (Grandin, 1986), goats (Lager, 1998), and sheep (Mellor, 2004) can be readily taught to cooperate during certain procedures that would otherwise trigger data-confounding stress reactions.

Do animals remember us?

It will be of no surprise to many caretakers that rats recognize familiar people. One caretaker remarked: "When I go on holiday, the new caretaker often tells me my rats are not as calm as I make them out to be." In a study investigating whether rats remember handlers, Davis et al. (1997) found that after one 10-minute handling session, 24 of 26 Long Evans rats correctly chose the handler with whom they had previous contact. Rats prefer contact with a person with whom they are familiar and with whom they had positive experience versus an unfamiliar individual.

Primates are an excellent example of how well an animal remembers us once we have built a trust relationship with them. Augusto Vitale (2011) shares his story about a strong bond he formed with a tufted capuchin monkey, named Cammello. Cammello was a very eager monkey, who readily solved many experimental tasks. Augusto had built a bond with this monkey, but didn't realize that the monkey had also bonded with him, until he returned to the monkey colony after a 20-year absence: "Cammello rushed to greet, embrace and groom me for about 5 minutes."

Naming animals

Naming animals raises many emotions in people; a name can strengthen a bond we feel towards an animal, but naming animals in a scientific context may be considered inappropriate (Baumans et al., 2007), as it personalizes them. Many care staff will strongly disagree with this attitude and firmly believe people should have deep feelings for the animals in their care (Baumans et al., 2007, Cruden, 2010). In the LAREF discussion summarized earlier, several participants mentioned that they give names to the animals in their charge or to the animals they study. Giving names to animals can be a reflection of empathy and is a useful tool to quickly recognize individuals.

Sullivan (pictured below) was one of three friendly male ferrets at a facility where the author used to work. Sullivan and the others (Felix and Gilbert) loved to come out of their cages, chase toys, and snuggle with the caretakers. This was both great enrichment for the ferrets and the staff, who would call for them by name.

Naming animals in the research laboratory is possibly one of the most powerful tools to create a bond. It personalizes them and creates a persona. An animal with just a number or no identification can be viewed dispassionately, as a mere receptacle, whereas a named animal is viewed as a living, sentient being who must be treated with compassion.

Does the bond with animals affect research data obtained from them?

Developing a bond with animals in research laboratories and knowing their fate can be an extremely difficult situation, emotionally, for the caretakers to come to terms with (American Association for Laboratory Animal Science, 2001). In spite of this emotional cost, in a survey of 81 UK-based animal technicians, 70% agreed that people had to love animals in order to do their often emotionally draining work (Cruden, 2010). It is undeniable that, at the very least, kindness and concern for animals are desirable characteristics of anyone involved in animal research (American Association for Laboratory Animal Science, 2001). It is important to note that a strong bond can be formed with rodents just as much as it can be formed with larger species such as primates and dogs. Often this fact is overlooked when people observe the paradox of caring about animals while working with them in a research environment.

Wolfle (1996) made it very clear that stress leads to profound physiological and behavioral changes that can increase the variability of the data and decrease the reliability of the results. In order to control stress, the caretaker must strive to develop an affectionate bond with all animals in his or her charge (Wolfle, 1987). This bond conveys to the animal a quiet sense of assurance, from which coping strategies can be developed for dealing with other stressful aspects of the laboratory (Wolfle, 1987). Rather than compromising research, these human-animal bonds should be considered the very foundation of scientifically sound research methodology. If we achieve this aim we will refine the very core of laboratory animal experimental design.

Bayne (2002) elaborates that the human-animal bond is not just a benefit for the animals but also for the staff involved; it can be an enriching and rewarding experience for both. Coppola et al. (2006) found that the a greater amount of human interaction with shelter dogs led to a lower cortisol level in the saliva, suggesting that more quality time spent with laboratory dogs will lead to less stressed animals. The European Guidelines recommend that frequent contact should be maintained so that the animals become familiar with human presence and activity (European Economic Community, 1986). Where appropriate, time should be set aside for talking with, handling, and grooming the animals.

With ever-increasing understanding about the value of the human-animal bond, one can hope there may come a time when its importance will be recognized across all research institutes, and we will all be able set aside quality time to spend with the animals in order to foster trust-based relationships—and just for the pleasure of being with them.

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Animal Welfare Institute Policy on Research and Testing with Animals

Research must not be conducted on animals unless, at minimum, the methodology fulfills the three "Rs" of Russell and Burch, including the following:

1. The animals are maintained in an optimum, species-appropriate environment.
2. The animals are under the care of professionally trained, compassionate personnel.
3. The animals' pain, physical discomfort, maladaptive behaviors, fear and anxiety are prevented or at least minimized by considerate and scientifically sound experimental design and appropriate use of anesthetic, analgesic or tranquilizing drugs.

Detailed policy

1. All institutions that conduct research and testing with animals must refine the research methodology and reduce and seek to ultimately replace animals wherever indicated and possible. These efforts should be supported and funded by both the research-funding agencies and the research institution's administration.
2. If alternative yet equally effective methods of experimentation or testing are available, they must be used in preference to any experiment conducted with an animal, particularly an experiment that is likely to cause pain, fear or distress.
3. Any experiment or test that inflicts trauma should be conducted with a fully anesthetized animal. If the procedure causes life-threatening injury, the animal should be euthanized following the procedure and before regaining consciousness.
4. If an animal is subjected to surgery from which he or she is expected to survive, a pre-planned pain evaluation and pain management schedule must be developed. This schedule must account for overnight and weekend hours. The pain evaluation must contain specific signs, behaviors or physical parameters to be measured in the animal. Staff must ensure adequate and timely administration of pain-relieving medications until the animal has recovered and is no longer in observable discomfort.
5. Professional staff must be available at all times—day and night, weekends and holidays—to care for the animals. The staff must make rounds for the purpose of ascertaining the state of each animal's health and well-being. The staff must be trained and authorized to dispense pain-relieving or tranquilizing drugs as may be necessary. While it may be a standard operating procedure to phone the investigator or director regarding such events, this action must not delay the provision of relief for the animal. Nursing care must be provided to all animals following surgery or other injurious interventions and to animals with chronic pathological conditions.
6. Staff must be compassionate and well trained. Ongoing training regarding best practices must be provided. The staff must be observant and empowered to make its observations known to the director of the laboratory, veterinarian or another trained individual duly authorized to make animal welfare or humane endpoint decisions. For example, a moribund animal should be euthanized. An animal who is suffering should be—depending on the situation and the nature of the work—anesthetized or sedated and given supportive care such as fluids, soft food, and custom bedding and/or otherwise treated to alleviate suffering, or the animal should be euthanized.
7. Housing for animals in research must provide sufficient space and materials to permit the expression of basic species-specific behaviors, including species-typical walking and stretching, foraging, retreating to a safe/sheltered place, burrowing and gnawing (rodents), climbing, perching and swinging (nonhuman primates), perching and scratching (birds), and rooting and wallowing (pigs). Social animals must be housed with one or several compatible conspecifics to address their biological need for companionship.

8. The great majority of animals in experimentation and testing are purposely bred for sale to research facilities. This is the preferred method of acquiring animals. These animals must be raised in facilities whose standards of housing and care are equal to or better than those described herein for research laboratories. Following the legally mandated waiting period, dogs and cats at municipal pounds may be donated to veterinary schools where surgical training to conduct spays and neuters are done or other treatments are performed that are intended to facilitate adoption of the animals. After recovery, the dogs and cats should be returned to the pound where it is hoped that adoptive homes will be found for them.
9. Only noninvasive research of direct benefit to the species' own survival may be conducted on threatened or endangered species.
10. Euthanasia must be considered a major responsibility. Staff carrying out euthanasia must be well trained, efficient in performing the procedure, and empathetic to the animals. The primary concern must be the animals. The location for conducting the euthanasia should be selected so as not to increase anxiety and fear. The method of euthanasia that is selected should ensure the quickest death possible. No animal should be discarded without monitoring him or her long enough after death to ascertain rigor mortis.
11. Journals should expand the materials and methods section to include information regarding animal housing conditions, bedding type, enrichment, refinement, and details of supportive or analgesic care. The only information in most published articles is the species (or strain of rodent), sex and age—thus making it impossible for concerned scientists to find details needed to confirm sound methodology and trustworthiness of the research data and statistical results.
12. Animals should be permitted to retire after termination of their assignment(s) to research, testing and education. The funding agency and research institution should earmark funds for the life-long retirement of these animals.

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