COMFORTABLE QUARTERS *for Laboratory Animals*

Animal Welfare Institute

Introduction

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Is it really necessary to provide animals in research institutions with comfortable quarters? Yes, species-adequate housing and handling conditions are not only a safeguard for the well-being of the animals but also a prerequisite for sound scientific methodology. Inadequacy of animal care can skew scientific findings and render the particular research useless (Donnelley and Nolan, 1990). It would, indeed, be naïve to rely on data collected from an animal:

- who experiences discomfort, frustration and/or distress resulting from spatial restriction [e.g., enclosure is too small to allow free, species-typical posturing and postural adjustment];
- who experiences discomfort, pain, fear, anxiety and/or distress resulting from enforced bodily restraint [e.g., immobilization during procedures]; or
- who experiences depression and frustration resulting from the inability to show species-typical behaviors [e.g., social animals kept in barren single-cages/stalls].

These experiences are reflected in an animal's physiological, psychological and behavioral responses to an experimental situation. The responses, however, differ from animal to animal because the experience itself is subjective. It is impossible to do truly "scientific" research under such methodological conditions because the data collected are influenced by unaccounted-for extraneous variables such as distress, fear, anxiety, discomfort, depression and boredom. "To demonstrate any experimental response against such a variable background generates a requirement for greater animal usage if the result is to be statistically valid" (Home Office, 1989, p. 8). "Good husbandry minimizes variations that can modify an animal's response to experimentation" (National Research Council, 1985, p. 11), thereby allowing the use of fewer animals giving equally valid results (Russell and Burch, 1959; Brockway et al., 1993; Chance and Russell, 1997). It is a fundamental scientific principle that all variables that have not proven to be insignificant be controlled in order to assure a sufficiently high degree of accuracy and reproducibility of the research findings. "If a researcher, through carelessness or ignorance, should use more animals for a project than is necessary, it must be considered unethical" (Öbrink and Rehbinder, 1999, p. 122).

Comfortable Quarters for Laboratory Animals offers suggestions and recommendations for how extraneous, husbandry-related variables can be minimized or avoided thereby maximizing the research animals' well-being and reducing the number of subjects required to obtain reliable research data. The basic conditions for the provision of comfortable quarters are outlined in regulations and professional guides:

Primary Enclosure

"Proper care, use, and humane treatment of animals used in research, testing, and education…require scientific and professional judgement based on knowledge of the needs of the animals.…A good management program provides the environment, housing, and care that…minimizes variations that can affect research.…The environment in which animals are maintained should be appropriate to the species.…Animals should be housed with the goal of maximizing species-specific behaviors and minimizing stress-induced behaviors. For social species, this normally requires housing in compatible pairs or groups. …At a minimum, an animal must have enough space to turn around and to express normal postural adjustments …and must have enough clean-bedded or unobstructed area to move and rest in.…Space allocations should be re-evaluated to provide for enrichment" (National Research Council, 1996, pp. 8, 22, 25 & 27).

Handling Procedures

"Handling of all animals shall be done as expeditiously and carefully as possible in a manner that does not cause …behavioral stress, physical harm, or unnecessary discomfort" (United States Department of Agriculture, 1995b, p. 21-22). "Restraint procedures should only be invoked after all other less stressful procedures have been rejected as alternatives.…Physiological, biochemical and hormonal changes occur in any restraint animal…and investigators should consider how these effects will influence their proposed experiments" (Canadian Council on Animal Care et al., 1993, p. 95). "To reduce the stress and pain of laboratory animals, nontraumatic restraining techniques must be taught.…We believe that teaching of procedural skills is crucial for maintaining high research standards within the laboratory" (Schwindaman, 1991, p. 30). "Many dogs, nonhuman primates…and other animals can be trained, through use of positive reinforcement, to present limbs or remain immobile for brief procedures" (National Research Council, 1996, p. 11).

Animal Care Personnel

"The behaviour of an animal during a procedure depends on the confidence it has in its handler. This confidence is developed through regular human contact and, once established, should be preserved.…All staff, both scientific and technical, should be sympathetic, gentle and firm when dealing with the animals" (Home Office, 1989, p. 16-17).

Providing animals in research institutions comfortable, i.e., humane quarters is not only a scientific but also an ethical obligation. After all, the caged animal is completely at the mercy of the investigator. To merely "use" animals for personal gain [e.g., promoting one's academic career] or for perceived benefits for people [e.g., developing treatments of diseases] without paying proper attention for their safety and well-being is ethically not acceptable. To show concern for the well-being of research animals, however, may stigmatise an investigator as being "scientifically soft" even though "awareness of actual and potential stress and distress among animals in whatever situation should not be regarded as subjective but as a sound scientific base for the study of animals. Whether an observer 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).

The chapters of the new edition have been written by animal care personnel, scientists and veterinarians who have demonstrated an active commitment to the humane and scientifically acceptable housing and handling of laboratory animals. In our invitation letter we have asked each author to:

- outline the species-typical characteristics of the species you are dealing with,
- make recommendations on how these characteristics can best be addressed in the research institution,
- make the well-being of the animals an uncompromising priority of your chapter and
- provide supportive references for all statements.

In the United States more than 14 million animals are used annually in research institutions. Only approximately 10% of these animals are regulated under the *Animal Welfare Act* (United States Department of Agriculture, 2000). The remaining 90% are either not considered at all [cold-blooded animals] or explicitly excluded [rats, mice, birds] in the regulatory definition of the term "animal" (United States Department of Agriculture, 1995a, p. 1), and they are, therefore, exempt from legal protection to "insure that animals intended for use in research facilities…are provided humane care and treatment" (Animal Welfare Act, 1985, p. 1). We see no scientific, ethical or logical justification for this seemingly arbitrary discrimination. Since rats and mice far outnumber all so-called "true animals" taken together, their inhumane care and treatment causes much more suffering and affects scientific findings in a much more pervasive manner. We feel that rats and mice, but also birds and cold-blooded animals, such as reptiles and amphibians, deserve the same consideration as other animals legally do, and we have therefore included chapters specifically addressing their needs for well-being in the research institution.

This is the ninth edition of *Comfortable Quarters for Laboratory Animals*, which was first published in 1955 for free distribution by the Animal Welfare Institute. May the recommendations set forth in this book serve as an inspiration to all those who are committed to safeguarding the well-being of research animals and the integrity of sound scientific methodology.

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Comfortable Quarters for Mice in Research Institutions

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Before describing how we can keep mice in more comfortable housing, it is worth briefly revisiting the reasons why this should be attempted. Each year many millions of mice are used throughout the world in research institutes. As part of this process, historically it has been the norm to breed and house mice under highly standardised conditions, aiming to reduce variability in responses and the data from subsequent research. This has meant that laboratory housing for mice is typically small, barren and monotonous. For some time, it has been questioned whether such housing systems compromise the welfare of the inhabitants. There is now convincing evidence that standard laboratory housing does indeed result in behavioural and physiological responses indicative of animal welfare compromises. Perhaps of greater importance is recent evidence that housing animals under such conditions affects the animals so fundamentally (Prior and Sachser, 1995; Prusky et al., 2000; Würbel, 2001) that concerns are being expressed about the validity of the data and its applicability to other circumstances. This calls into question the very reason for the animals being housed in these conditions in the first place.

There are compelling welfare and scientific reasons why we should house laboratory mice under conditions more suited to their own species-specific needs. These two factors are addressed separately below, with an emphasis on how they are inter-related.

Welfare Reasons for Providing Comfortable Quarters

Laboratory housing for mice has evolved from designs that were initially primarily concerned with economics, human convenience and extreme standardisation of the environment. This means that in current systems, the behavioural requirements of the animal are largely not catered for, other than the basics of feeding and drinking (Figures 1a & b). When given the opportunity, laboratory mice show a diverse behavioural repertoire: they seek a wide variety of foods, are very active physically, form complex social organisations, build tunnels and construct nests (Jennings et al., 1998). All these behaviours are thwarted by standard husbandry and housing. Housing systems should allow animals to perform most natural behaviours (e.g., "The Five Freedoms"; Farm Animal Welfare Council, 1997) to avoid compromises of welfare. In addition, if animals are prevented from performing behaviours for which they have a strong motivation, this can lead to suffering and adverse mental states such as frustration, depression and anxiety (Dawkins 1990; Duncan, 1992; Sherwin and Nicol, 1998). Certainly, conventional standard laboratory housing prevents many natural and highly motivated behaviours [e.g., nesting, tunnelling, extensive locomotion]. As a result, mice in laboratory conditions frequently exhibit so-called abnormal behaviours, for example stereotypies (Würbel et al., 1996; Nevison et al., 1999a), indicating that mice experience chronic frustration when placed in conventional, non-enriched cages (Sherwin, 2000). Furthermore, the sensory capabilities of mice have rarely been considered in laboratory housing and husbandry design. Mice have sensory modalities that are sometimes very disparate to humans [discussed below]. Our historical ignorance of these sensory capabilities means that standard housing generally does not take into account the perceptions of mice. This is potentially the equivalent to rearing animals under conditions of sensory deprivation or interference [e.g., olfactory "white noise"] with all the concomitant compromises in welfare (Cummins et al., 1977; van Praag et al., 2000).

Scientific Reasons for Providing Comfortable Quarters

Animals reared in barren conditions are generally more sensitive to environmental perturbations or differences between laboratories. Therefore, when mice reared in conventional, barren cages are moved to a new laboratory, their behaviour might not be representative of "normal" responses. Moreover, there is growing evidence that the minimalistic environments of laboratory mice impose constraints on behaviour and brain development such that many studies using these animals may have little external validity, particularly in neuroscience studies.

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Studies may achieve good internal validity [i.e., reduced variation between animals in the same experiment in the same laboratory], but there might be increased variation between animals undergoing the same experiment in different laboratories (Crabbe et al., 1999). This diminished external validity calls into question the reason for keeping mice in conventional, barren cages. It can arise in three ways (Würbel, 2001):

1.Neurophysiological changes. It is well established that in rats, barren environments, compared to enriched ones, result in decreased numbers of brain neurones, synapses and dendritic branches, especially in the cortex and hippocampus. This results in impaired learning and memory (Rosenzweig and Bennett, 1996; van Praag et al., 2000). It has been argued that such effects suggest animals reared under standard laboratory conditions experience something akin to sensory deprivation (Cummins 1977; van Praag et al., 2000).

2.Chronic thwarting of behavioural response rules. Animals respond largely according to evolved rules that depend on specific environmental features. If these features are absent, the animal might display inappropriate behaviour. For example, gerbils will develop stereotyped digging if they do not have a suitable shelter. This stereotyped behaviour is prevented if they are presented with a shelter, but only if the shelter has a tunnel-shaped entrance (Wiedenmayer, 1997). Thus, barren cages can limit the opportunity for animals to develop appropriate behaviour with consequences for later studies in which it is assumed the animal is behaving "normally." In addition, chronic thwarting of behavioural responses can lead to stereotypies, associated with functional changes in the dorsal basal ganglia and a general tendency to perseverity [inability to be flexible in behaviour] (Albin et al., 1989; Ridley, 1994; Hauber, 1998). This could easily lead to scientists using an animal model that is fundamentally flawed.

3.Mismatch between postnatal and adult environment. The barren, monotonous environment in which mice are reared very often conflicts with the more variable life of mice undergoing research later in adult life. This can have a profound influence on the validity of the research. Laboratory-reared mouse pups are less stimulated than their wild counterparts as the laboratory-mother leaves the nest less frequently and for only short periods because food and water are provided nearby. However, if pups are handled for just a few minutes each day, the mother increases visits to the nests and grooms the pups to a degree that is thought to more accurately represent what is adaptive in the wild. As a consequence, pups who have been handled and thus better attended to by the mother [which might be encouraged by cage design and enrichment rather than handling] show reduced behavioural and endocrine responses to stress (Würbel, 2001). This could manifest as a reduced sensitivity to procedures causing pain, distress or suffering. In addition, barren environments at an early age can lead to improper development of the senses, e.g., vision (Prusky et al., 2000), with obvious consequences for studies requiring "normality" of these senses. The mismatch between postnatal and adult environment caused by standard laboratory housing is likely to have considerable, multifarious implications for research conducted on animals housed in these systems.

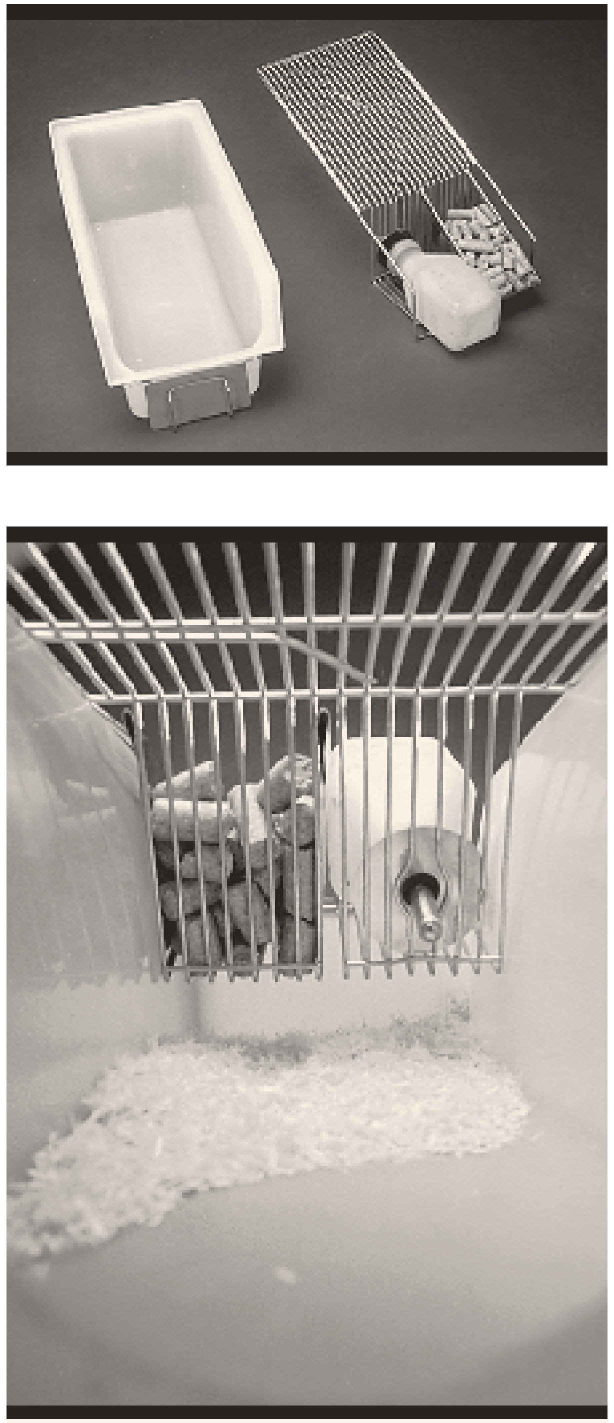

Figure 1a, b. A human perspective and a mouse perspective of a standard laboratory cage. The inside view of a standard cage shows this design caters little for the species-specific characteristics other than feeding and drinking.

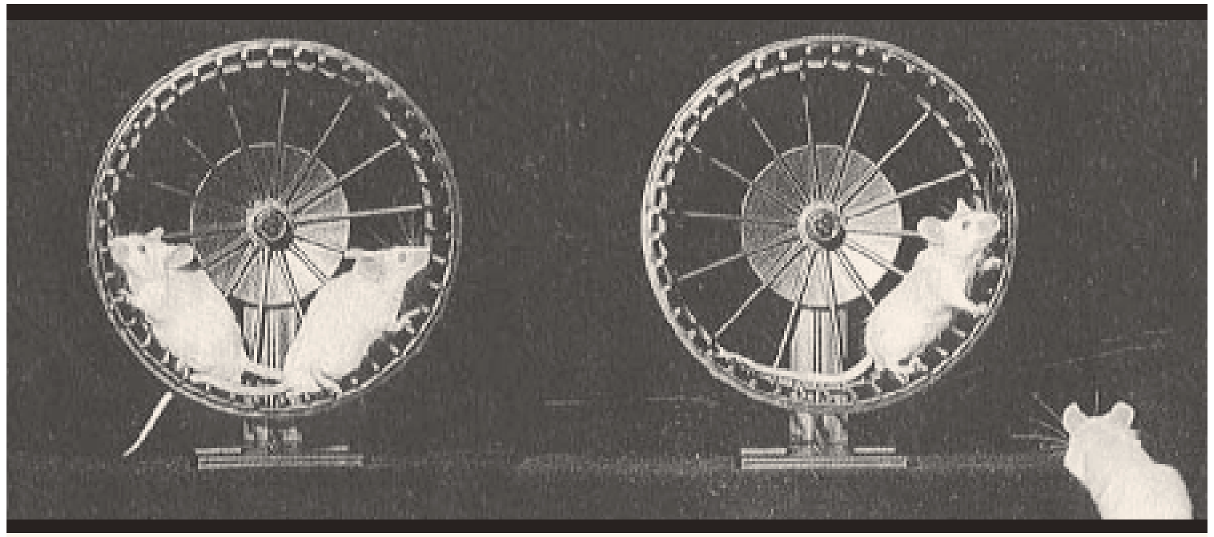

Figure 5. Running wheels are suitable to promote exercise in captive mice.

but in non-breeding animals can also help to regulate temperature and light levels and to hide and retreat from cage-mates or other threatening stimuli. There is considerable evidence that mice are strongly motivated to build nests (Blom 1993; Sherwin 1996, 1997; van de Weerd et al., 1998), indicating that this activity fulfils one of their most fundamental behavioural needs.

Several products are commercially available, but hay, straw, shredded paper, wood chips and paper tissues are all useful. Paper towels can be left on the cage lid for the mice to energetically drag through the bars and chew into pieces to build a nest. If several materials are available, mice will generally build a composite nest (Figure 4). Although providing nesting material is easily and inexpensively achieved, it is one of the best and most versatile enrichments for mice kept in research institutions. Suitable nesting supply should be provided for all animals, although for cages with new-born young, materials that might entrap legs should be avoided [e.g., cotton wool, wood wool, shredded paper] and materials that absorb moisture as these can stick to wet pups and cause dehydration.

Increased Activity

Mice are extremely active animals, yet the physical dimensions of a standard sized cage allow mice to move only a few body lengths in any one direction. This spatial restriction, in conjunction with a plentiful supply of food nearby, means that mice can quickly become overweight with a subsequent reduction in life-span. They demonstrate a strong motivation to gain access to additional space to that provided by a standard laboratory cage, even when this provides no further resources or enrichment (Sherwin and Nicol, 1997); this could be interpreted as the mice having a strong urge to escape standard laboratory conditions!

One method of providing an opportunity for increased activity is a running wheel (Figure 5). There is much evidence to suggest that providing a running wheel is of great benefit. Mice will work hard to gain access to a wheel, they prefer a wheel to an extended surrogate tunnel system, and there are many physiological and behavioural advantages related to welfare. Mice sometimes appear to play in running wheels. For example, they will grip the rungs of the wheel until they are carried around and around by the wheel's momentum. They will turn motorised wheels on and off. It has even been reported that mice prefer wheels that have been made into irregular shapes, or include hurdles to jump over (Sherwin, 1998 a,b).

Other methods can be utilised to encourage increased activity, even within the confines of a relatively small cage. Simple activity discs can be made relatively easily and cheaply (Figure 6; Animal Welfare Institute, 1979). Commercial pet companies manufacture "activity dishes," which resemble a miniature satellite dish set at an angle to rotate about a central axis (Figure 9). Climbing frames, ropes, pieces of string or chains all allow mice to climb. In addition,

Figure 6. Activity discs can be made relatively easily and cheaply (photo by Ernest P. Walker, 1979).

the bars of the cage-lid are used prodigiously; if taller cages are used, enrichments allowing access to the lid should be provided. For this reason, amongst others, cages with solid tops are not recommended.

Tunnel-building

Many wild rodents build complex tunnel systems (Ellison, 1993; Schmid-Holmes, 2001). These are used to escape predators (Blanchard et al., 1995) and presumably for other comfort factors including thigmotaxis. Laboratory mice who have never had the opportunity to dig tunnels will build these within a few hours if a suitable substrate is provided (Sherwin, personal observation; Figure 7). Unfortunately, providing mice with the opportunity for tunnelling can make them rather difficult to catch although they will often sleep in an attached cage that leads to the tunnelling substrate. However, if regular handling is not required, or naturalistic behaviour is desirable, several centimetre deep, suitable substrate [e.g., damped peat with rocks or fibrous bedding to support the tunnel system] provides for almost instantaneous digging and some very entertaining mouse behaviour. Wood chip bedding might be a suitable compromise as it allows mice to perform digging behaviour and seek a darker environment but does not allow them to totally escape detection from human concerns. Surrogate burrows can be offered in the form of plastic tubes designed for pet rodents; several types are available commercially. Laboratory mice seem to gain a great sense of security in these tunnels even when they are transparent; they often appear completely oblivious to nearby human presence. Providing tubes as tunnels can also make catching the mice a little difficult, although the tunnels can usually be separated into smaller sections and the one containing the mouse placed into the cage he or she is being transferred to; the mouse then usually walks out of the tube within a few seconds. Alternatively, if there are short tubes, mice use these as retreats and run into them during attempted capture. The tube containing the mouse is then easily transferred elsewhere, or the protruding tail of the mouse used for quick and easy handling, which also reduces stress caused to the mouse.

As described before, commercial pet companies produce "mazes" for pet rodents that might provide a practical surrogate tunnel system for laboratory mice in some situations.

Chewing/gnawing

Mice will readily chew on a variety of objects and should be provided with the opportunity to express this behaviour. Such chewable objects might include cardboard tubes, softwood blocks, old plastic water bottles, hay, straw, etc. (Figure 8). Cardboard tubes are particularly versatile as they also provide opportunities for shelter, climbing and manipulation.

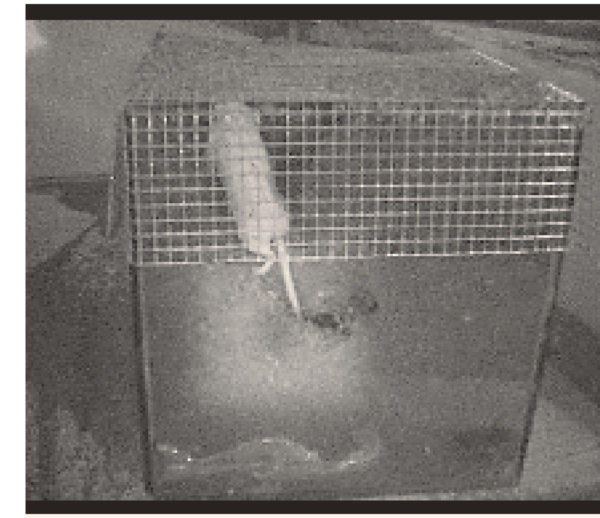

Figure 7. Laboratory mice who have never encountered deep substrate will readily dig tunnels when given the opportunity.

Figure 8. This enrichment is advertised as a wooden chewing block, but its design allows it to also be used as a nest/shelter (the mice drag paper into it) and a climbing object.

Thermoregulation

Some rodents prefer cooler ambient temperatures in the dark phase and warmer temperatures during the light phase (Gordon, 1993). This suggests that a diurnally changing temperature might contribute to improving the animals' comfort. Of course, providing suitable nesting material is likely to circumvent the preference for changing ambient temperature and also provides the opportunity for other

Figure 3. Lister hooded rat nursing her ten-day-old pups in "cover position."

They will sleep in a heap or separately depending on the ambient temperature. They are easily aroused and do not normally sleep for long periods without waking intervals. Mature animals settle down to sleep by tucking their heads between the forepaws while in the quadrupedal position. As they move into a deeper sleep phase, they suddenly keel over onto a side, typically at full length with tail extended. To assume this natural sleeping posture an adult rat requires a floor space of 15 x 35-45 cm. Rats sleep in a curled position only when chilled. When waking up, they stretch and yawn with fully opened mouth while the head is thrown back and the forelegs extended. When a rat stretches, one forepaw goes forward of the head while a back foot is stretched out beyond the tail base, the tail itself being arched; then the feet are reversed. Finally, the animal shakes itself.

Rats will progress by creeping when they are nervous, insecure, or alarmed. In this mode of locomotion the animal flattens his/her belly against the floor and shoves the trunk forward by "paddling" with laterally extended limbs. When a rat is walking normally, the tail is carried off the ground straight out behind the trunk. Any other pattern is distorted and indicative that the animal is kept in a too small enclosure. The running pace is probably three or four times longer than the walking pace. When a rat is running, the tail is carried straight out behind the body with the tip of the tail upturned. Juveniles and females in estrus spend conspicuous amounts of time running around. Rats can achieve considerable speed, especially in a panic-stricken dash. When merely exploring a new place they lope along at a much more modest speed.

The motivation to forage is very strong in rats, and they will readily work for the retrieval of food in the presence of freely accessible identical food (Neuringer, 1969; Carder and Berkowitz, 1970; Hothersall et al., 1973). They will eat powdered or mushy food from a dish or from the floor, but their species-specific habit is to secure a piece of food in their teeth and carry it to a suitable spot where they adopt a squatting posture and transfer the food to the forepaws. Holding the food in their paws, they nibble gently at it; if they do not like the taste they drop it immediately. The opportunity to gnaw is an essential physiological and behavioral need for rats. If they are not given the chance to regularly gnaw, their front teeth overgrow and make it more or less impossible for them to eat at all or to engage in grooming.

Rats have a spontaneous fear of people and avoid being handled. Handling can be a powerful stressor for them (Brown and Martin, 1974; Kvetnansky et al., 1978; Berkey et al., 1990; Briese and Cabanac, 1991) introducing uncontrolled variability into research data (Shyu et al., 1987; Brockway et al., 1993; Claassen, 1994). Rats are also very sensitive to environmental disturbances. Even ordinary animal husbandry procedures such as moving a cage to a different area or moving animals to a clean cage can induce transient, but significant, physiological and behavioral changes that may confound experiments conducted shortly thereafter (Gärtner et al., 1980; York and Regan, 1982; Saibaba et al., 1996; Duke et al., 2001).

The natural defense of rats who experience threat is not to hang about but to run and hide, and if possible huddle with other conspecifics in a safe place. Being placed on an open surface is an especially threatening situation (cf., Latané, 1969).

Minimum Recommendations for Rat-adequate Housing and Handling Conditions

The ethogram provides a base from which the behavioral needs of a species can be derived and that allows one to make recommendations regarding the minimum space and caging conditions required by the animals to satisfy those needs and experience a state of behavioral and physical well-being.

The cage in which a mature rat can adopt species-typical postures and stances and can carry out essential activities has to measure between 35 x 25 x 18 [height] cm for the smallest females and 50 x 30 x 30 [height] cm for the largest males. Table 1 lists the minimum space requirements by sex and body weight. It must be emphasized that young animals require more space, relatively, for play activities. Therefore, they should not be allocated less space than is appropriate for the smallest females [35 x 25 x 18 cm].

Rats of any age should not be caged singly or in large groups. For adults the group should not be more than six animals, for juveniles not more than ten animals. Rats kept in larger groups tend to be too aggressive and are more prone to disease. Pair-housing is probably the optimal alternative both to single-housing and to group-housing (Heath, 1999). Separation from conspecifics is a distressing situation for rats leading to significant physiological alterations (Ehlers et al., 1993; Young et al., 1996; Lawson and Churchill, 2000). Individually caged animals are susceptible to stress (Hurst et al., 1997), which again jeopardizes the validity of research data collected from such animals (Pérez et al., 1997). Rats show more pronounced stress-like changes in behavior and cardiovascular function during common husbandry and experimental procedures when they are housed alone than when housed with another rat (Zammit et al., 2001). If an

BODY WEIGHT OF RAT [1G=0.035 OZ.]	MINIMUM FLOOR AREA CM² (IN.²)		MINIMUM HEIGHT CM (IN)
	FOR 1-3 RATS	FOR AN ADDITIONAL RAT	
male up to 150 g female up to 140 g	900 (140)	300 (47)	18 (7)
male 150-250 g female 140-170 g	1200 (186)	450 (70)	20 (8)
male 250-450 g female 170-310 g	1500 (233)	600 (93)	22 (9)
male 450-900 g female 310-615 g	1800 (279)	800 (124)	26 (10)
male over 900 g female over 615 g	1800 (279)	1000 (155)	30 (12)

Table 1. Minimum space recommendations for laboratory rats.

animal has to be single-caged for veterinary reasons provision must be made that he/she can keep visual and auditory contact with other rats to buffer the stress associated with isolation (cf., Latané and Glass, 1968).

The main food staple for laboratory rats is ordinarily a commercial high quality pelleted diet fed ad libitum. Hard pellets usually provide for sufficient gnawing. ***Natural food items, however, such as carrots, grain/seeds, and/or pieces of soft wood, are more species-appropriate items for gnawing.*** Rats should always have free access to them. Wooden gnawing blocks are attractive enrichment objects (Chmiel and Noonan, 1996) that not only reduce the incidence of stereotypic chewing of metal cage bars (Orok-Edem and Key, 1994) and make the animals less timid (Eskola and Kaliste-Korhonen, 1998) but are available with certificates of analysis, a particularly important aspect for toxicological studies (Robertson, 1999). Rats "want" to forage (cf., Neuringer, 1969), and they can easily be induced to "work" for their food by soldering metal plates over their food hoppers, so that only a small segment of the original area remains available. This method of "food restriction" is preferable to giving less food to avoid obesity. Rather than rapidly eating a reduced ration and feeling hungry for long periods, the animals work harder for their food, which enables them to burn more calories and eat throughout the day. This reduces the incidence of obesity and its associated disorders and also encourages more "natural" behavior patterns, both of which improve welfare (Wrightson and Dickson, 1999).

In order to provide rats a sense of security and options of breaking visual contact with each other during agonistic conflicts, it is recommended to add vertical barriers (cf., Anzaldo et al., 1994) and/or tubes—made of PVC or aspen wood (Mering, 2000)—in their cages. This offers the animals additional wall contact, tactile comfort, escape routes, and areas for exploration, thereby increasing cage complexity and the usable floor space of the cage. Evidence suggests that a more complex housing environment—in sharp contrast to the barren cage—buffers anxiety responses to potential stressors (Levine, 1985). ***A well-designed cage provides a distinctive sheltered nest area away from the feeding location.*** Rats with access to an appropriate shelter are more explorative and less timid than those in barren cages (Townsend, 1997). Nest-boxes of opaque or semi-opaque materials are particularly suitable shelters (Manser et al., 1998). Ideally, rats should always have access to one cage section that is covered with a black perspex screen serving as dark-and-sheltered sleeping and hiding area and another section serving as living area (Figures 4; cf., Wrightson and Dickson, 1999). The living area section should be covered with a wire lid for gymnastics.

It should go without saying that solid floors are much more appropriate for the feet of rats than wire floors, which impact the feet in a biologically abnormal manner (Grover-Johnson and Spencer, 1981) and may cause discomfort, pressure sores, and pain. They may also cause chilling even in a warm room. While rats housed on grid or mesh floors

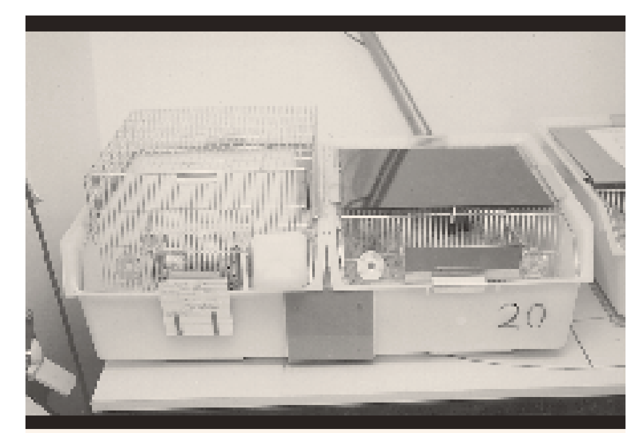

Figure 4. The ideal double-cage arrangement: The right cage section is covered with a black perspex screen and serves as dark-and-sheltered sleeping/nesting area. The left cage section serves as living area, which is covered with a wire lid for gymnastics.

housed animals should, therefore be at least 75 cm high and no less than 80 cm long. It should be 68 cm wide to allow the animal to comfortably turn around and change postures (Gunn-Dore, 1997).

Each cage should be provisioned daily with high-quality ***hay*** to promote the expression of foraging, playing, investigating and nesting behavior. The hay should be placed on the top of the cage so that the animal can spend some extra time retrieving it through the bars. There should also be at least one ***wooden stick*** [length approximately 10 cm; diameter approximately 2.5 cm] or other rabbit-suitable enrichment gadgets, such as brass wire balls triggering species-typical gnawing, chin-marking and playing (Huls et al., 1991; Gunn-Dore, 1999). Gnawing sticks have been used for a 2-year test period as effective enrichment objects for single- and pair-housed rabbits without noticeable hygiene and health problems (Brooks et al., 1993). It is a general idea at some facilities that rabbits need gnawing sticks to prevent their teeth from getting too long (Lindfors, 1997).

Single-caged rabbits who have access to hay and other enrichment objects show a reduction in stereotypical behaviors and a marked increase in their overall activity, relative to animals kept in barren cages (Gunn-Dore, 1997; Berthelsen and Hansen, 1999). Hay has proven to be particularly effective in reducing behavioral disorders and giving individually housed bucks something to do (Lindfors, 1997). The single-housed rabbit also needs a "safe" refuge to hide in alarming situations. A section of a PVC tube can serve as a substitute ***burrow*** meeting this requirement.

Cages should be designed in such a way that the rabbits are not restricted to grid or wire flooring—which is uncomfortable for the animals and very often results in sore hocks [ulcerative pododermatitis] (Kraus and Weisbroth, 1994)—but that they also have access to a ***raised solid-floor area***. This raised area offers a choice of resting sites, light gradients and a stimulus for exercise (Stauffacher, 1993; Gerson, 2000). The cages should be arranged at waist-height for easy access and cleaning. Multi-tier caging systems are not recommended because they do not allow the provision of uniformly distributed illumination (United States Department of Agriculture, 1991), a prerequisite to avoid variability of research data resulting from variable illumination in the cages (Bellhorn, 1980; Clough, 1982).

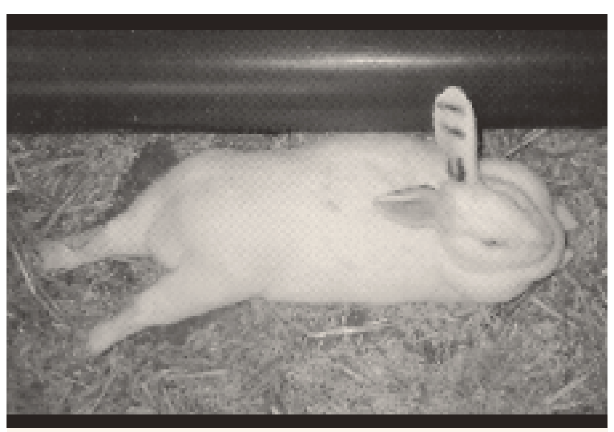

Figure 6. An adult rabbit is approximately 80 cm long when resting in typical rabbit-fashion (photo by Debbie Gunn-Dore, 1997).

Figure 7. Unsedated rabbits waiting to have blood samples taken. The rabbits are accustomed to travelling to and from their pens on these carts.

The Animal Care Technician's Role in Providing a Stress-free Environment for Rabbits

Although comfortable housing is important for the rabbits, much of the effort would be wasted if the other activities surrounding the rabbits were not also comfortable and non-stressful. In this respect, the animal care technician plays a vital role. The following are examples where technician/rabbit interactions are important.

Group-housed rabbits must be caught with a minimum of chasing. We can make use of the rabbit's natural tendency to hide when startled. In our case, the rabbits duck under the resting board (Figure 2) where they may be identified, picked up and handled in a gentle and skillful manner. Any dark hiding place will serve the same purpose, but a quiet, smooth approach is required. ***It is important not to startle the animal in his or her hiding place.*** Once the animals are used to being picked up, they may not even hide from a technician they know well. The anticipation of what is to happen after being caught plays a major role in the rabbit's behavior. Procedures carried out with the rabbits should be as free of stress as possible. Rabbits who are used to being treated with compassion and professional skill will not panic in anticipation of procedures (Figure 7). Carefully bundling a rabbit in a blanket and gently covering his or her eyes with a towel usually has a calming effect, even on a very agitated animal.

The traditional rabbit restrainer for taking blood samples is unnecessary if you provide good analgesia and some gentle handling. Blood sampling is least stressful if the subject is given a sedative and an analgesic. The added advantage is that the arteries and veins are dilated, making it easier to take the samples. Local anesthetics [e.g., EMLA cream] may serve the same purpose.

Rabbits have the potential of learning to cooperate rather than resist during procedures. It has been documented that they can easily be trained to cooperate during oral drug application, thereby avoiding the stressful gastric intubation procedure. The animals would stand with their paws on the front of the cages, protrude their faces from between the bars, and appear to beg for the sucrose-coated tip of the syringe containing the drug (Marr et al., 1993).

It is important that illness is recognized early in laboratory rabbits. This can be crucial because pre-emptive treatment for diseases like coccidiosis is often contraindicated. As a prey species, rabbits will disguise any signs of illness if they can. A reduction of food intake may be an early sign. It is useful to weigh the rabbits whenever they are handled, for example when blood samples are being taken (Figure 8). This allows early detection of inappetence. In addition, small quantities of treats, such as carrots, lettuce or leafy hay, may be used to check if the rabbits are still eating (Figure 9). Normally all members of the group will gather round the treat. A rabbit who hangs back may not be feeling well and should be looked at a little more closely. Personnel who regularly distribute treats are recognized by the rabbits who will often gather at the front of the pens at the sound of the treats bag. This is an elegant way to check all members of the group, a task that should be done at least once every day. Technicians quickly learn to notice subtle changes in behavior and so become aware of health problems. Special work time should be set aside for them so that they can pet their charges every day, thereby fostering a positive human-animal relationship (Home Office, 1989). ***The gentle touch provided by the technicians is as important as the physical environment in giving the rabbits a sense of security*** in the presence of humans who, in other circumstances may subject them to uncomfortable, perhaps even painful procedures. Gentle, frequent handling of rabbits buffers their fear response during stressful situations (Anderson et al., 1972; Kertsen et al., 1989). Rabbits who receive special attention from personnel [frequent handling, petting, playing, gentle vocalization] show a markedly increased resistance to certain pathological processes than subjects who receive no extra attention (Nerem et al., 1980).

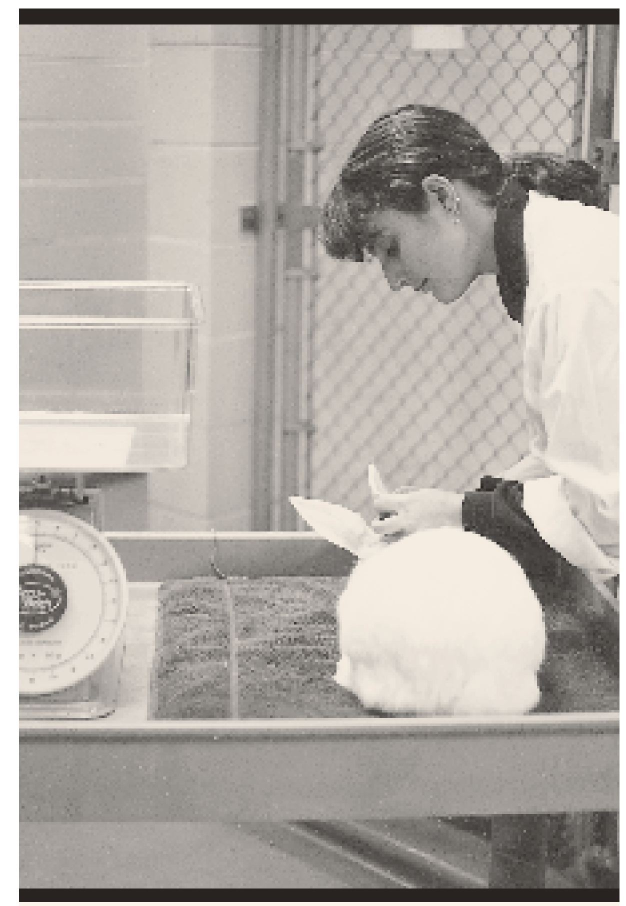

Figure 8. Regular, gentle health checks and weighing are important in monitoring the well-being of the rabbits and fostering a positive human-animal relationship.

Figure 9. Providing treats helps win the confidence and trust of the rabbits and allows the technician to check their appetite.

Figure 11a,b,c. Primates deserve fresh fruit or vegetables on a daily basis. It would be a waste of time to chop the produce for the animals; they have the time and they enjoy doing it themselves.

Branch segments of dead deciduous trees—red oak disintegrates into flakes that are so small that large quantities pass sewage drains without clogging them (Reinhardt, 1992a)—are perfect toys for primates, stimulating not only processing but also manipulative and play activities (Figure 12). They constantly change their form and texture due to wear and dehydration and, therefore, retain their stimulatory value (Reinhardt, 1989b; Eckert et al., 2000). Commercial toys lack the natural, ever-changing texture of wood; this is probably the reason why the animals quickly lose interest in them (Crockett et al., 1989; Line et al., 1989; Hamilton, 1991; Pruetz and Bloomsmith, 1992; Kessel and Brent, 1998), unless several different toys are offered and substituted regularly with new ones (cf., Paquette and Prescott, 1988; Weick et al., 1991). Access to a variety of manipulable objects seems to be particularly beneficial for capuchins and baboons, who exhibit sustained interest in them and respond with a significant reduction in abnormal behaviors (Brent and Belik, 1997; Boinski et al., 1999).

A major contention is the need for proper **illumination** in the caging arrangement of medium- and small-sized primates. In order to minimize housing expenses, these animals are traditionally kept in two-tier cages, with one row stacked on top of another. This doubles the number of primates that can be accommodated in one room, but involves serious implications for the individual animals. Those relegated to the lower rows are restricted to a terrestrial lifestyle, unable to withdraw in alarming situations and retreat to a safe place above the human predators who periodically capture them and subject them to distressing, or even deadly procedures. Moreover, the sanitation tray, which runs the length of the room beneath the upper tier of cages, reduces significantly the amount of light that can penetrate to the lower-cage tier (Schapiro et al., 2000b); “animals in the lower tier are thus relegated to a permanent state of semi-gloom” (Mahoney, 1992; p. 32). The cave-like living quarters of bottom-row caged animals is often so dim that caretakers routinely have to use flashlights to identify and inspect them (Figure 13). It has been noticed in marmosets that the housing environment of lower-row caged animals can be so poor that it results in markedly reduced fertility (Heger et al., 1986). Routinely rotating animals between bottom and top tiers (National Research Council, 1998) offers no solution to this problem. It merely “rotates” the problem by alleviating the situation for lower-row subjects, while aggravating it for the same number of upper-row subjects. At the same time it introduces the additional stress-variable associated with cage transfer (Mitchell and Gomber, 1976; Phoenix and Chambers, 1984; Crockett et al., 1993; Schapiro et al., 1997). Even if techniques can be developed to assure uniform illumination, the bottom-tier cage will remain a potential source of distress whenever personnel enters the room (cf., Kaumanns and Schönmann, 1997). In order to provide ethically and scientifically acceptable caging conditions, nonhuman primates must be housed in single-row cages to assure that (a) all animals receive the same quantity and quality of light, (b) all cages

Figure 12. Branch segments are perfect toys for primates. Constantly changing their texture and configuration due to wear, these wooden toys do not lose their stimulatory value over time.

are of sufficient height so that occupants are in a position to retreat above animal care personnel, and (c) all animals in the room can be adequately inspected.

Training nonhuman primates to cooperate during procedures is one of the most significant options of making life a little bit more bearable for laboratory primates. It not only challenges the animals’ high degree of intelligence, offers them—and the caregivers—some relevant distraction and eliminates data-confounding distress responses, but it also increases personnel safety by no longer giving the animals reason to defend themselves by means of biting or scratching during compulsory immobilization. To be forcefully removed from the familiar cage and subdued during painful husbandry and research procedures must, indeed, be a terribly frightening experience for a monkey or an ape. Research data collected from such an animal are tainted by the subject’s stress reactions (review: Reinhardt et al., 1995) and, therefore, have questionable scientific value (Figure 14). With gentle firmness, patience and positive reinforcement many primate species can be conditioned to work with—rather than against—personnel during common procedures such as transfer to a holding area (Goodwin, 1997; Bloomsmith et al., 1998), capture from the home cage (Reinhardt, 1992b), capture from the group (Reinhardt, 1990b,c; Kessel-Davenport and Gutierrez, 1994; Mendoza, 1999; White et al., 2000), blood collection (McGinnis and Kraemer, 1979; Reinhardt, 1991; Laule et al., 1996; Moore and Suedmeyer, 1997), blood pressure measurement (Smith and Ansevin, 1957; Mitchell et al., 1980; Turkkan, 1990), systemic injection (Spragg, 1940; Levison et al., 1964; Byrd, 1977; Priest, 1991; Reinhardt, 1992c; Figure 15a,b), urine collection (Kelly and Bramblett, 1981; Ziegler et al., 1987; Bond, 1991; Anzenberger and Gossweiler, 1993; Shideler et al., 1994), saliva collection (Bettinger, 1998; Bettinger et al., 1998), topical drug application (Reinhardt and Cowley, 1990; Segerson and Laule, 1995), oral drug application (Turrkan et al., 1989), semen collection (Brown and Loskutoff, 1998), insemination (Desmond et al., 1987), vaginal swabbing (Bunyak et al., 1992; Hernándes-López et al., 1998) and veterinary examination (Brown, 1998). The initial time investment in the training quickly pays off in: (a) a reduction of time required to obtain a sample, administer a drug or capture an animal, (b) a reduction of risks associated with defense aggression, (c) a reduction in the use of pharmacological restraint agents, (d) more reliable research data (Elvidge et al., 1976; Reinhardt, 1992c; Schnell and Gerber, 1997; National Research Council, 1998) and a more satisfactory relationship between handling personnel and research subject (Figure 16a,b,c,d).

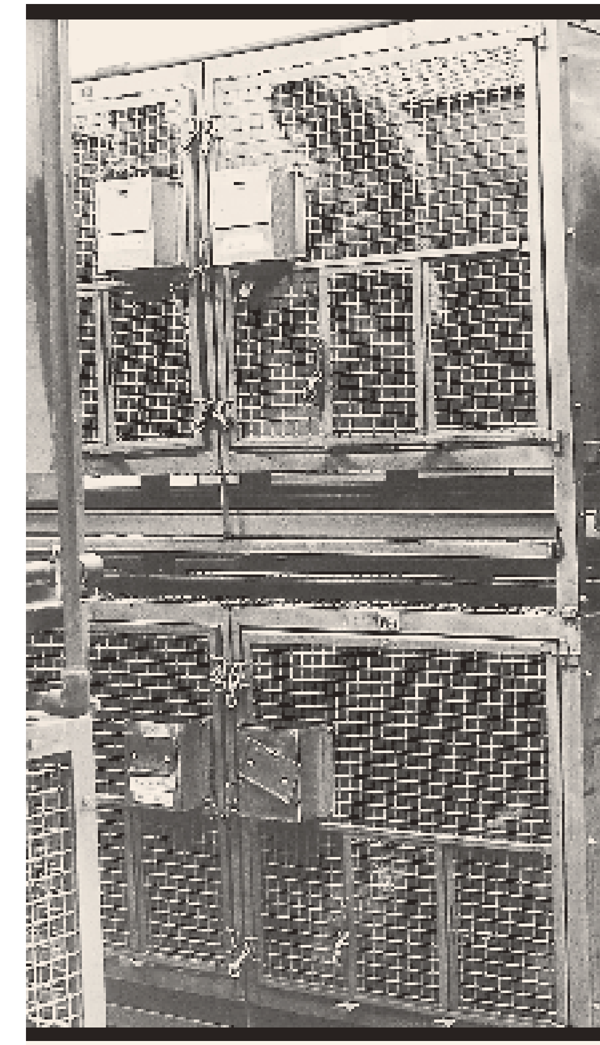

Figure 13. Even though it is the prevailing housing arrangement, the double-tier caging system is unacceptable both for ethical and scientific reasons.

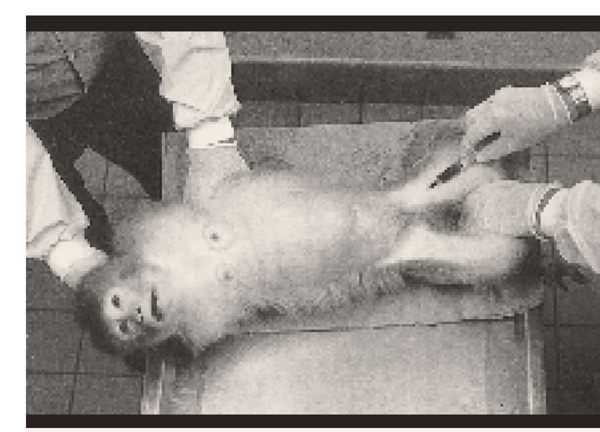

Figure 14. Scientific data collected from a subdued animal are skewed by the subject’s fear response.

Concluding Remarks

Providing primates in research institutions with primate-adequate housing and humane handling conditions is no sentimentalism. On the contrary, it is essential to employ such refined methodology in order to adhere to the very basic principles of good science. A primate who behaves like a primate and who is free of

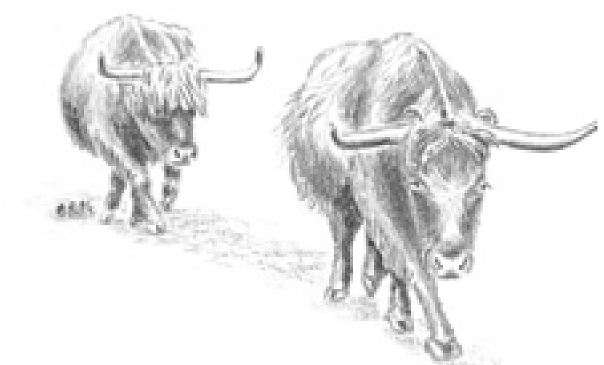

Figure 2. Cattle do not use their horns to injure each other. Subordinate animals avoid overt aggression by moving out of the way of dominant partners. Note the bossy look of the cow at left and the moving-away gesture of the subordinate cow at right (drawing by Annie Reinhardt).

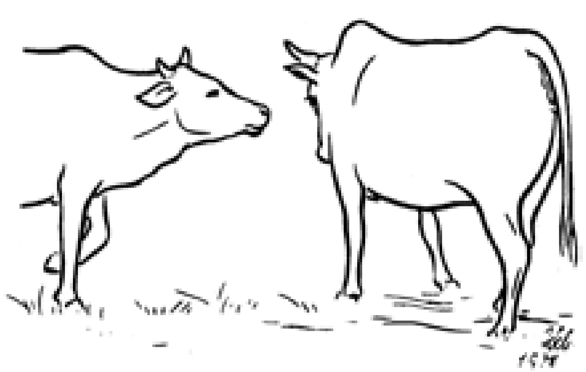

Figure 3. A strange cow [animal at left] submissively approaches another herd and is blocked from proceeding by a cow who displays the broadside-threat gesture (drawing by Ingrid Schaumburg).

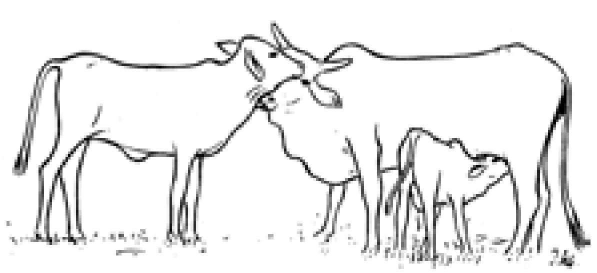

Figure 4. Cow *Aida* nursing her newborn calf while grooming her 13-month old daughter whom she had weaned four months ago. *Aida's* mean calving interval was 338 days; she produced 9 calves—who were all allowed to stay with the maternal herd beyond the age of natural weaning— during a test period of 7.5 years (Reinhardt, 1983a; drawing by Ingrid Schaumburg).

housing (Bouissou, 1970). Sufficient space is necessary so that subordinate animals can yield to dominant partners, thus triggering no overt aggression (cf., Figure 2).

Stable rank relationships are a prerequisite so that there are no undue social tensions or overt conflicts. A group's composition should, therefore, not be altered unless there is a specific veterinary reason. The members of a cattle herd know each other intimately and will show xenophobic behavior towards other cattle (Reinhardt, 1980; cf., Schloeth, 1961; Scheurmann, 1975; Figure 3). To introduce a strange cow into a herd is not a good idea!

A loose-housing system that is designed to meet the animals' social spacing requirements along with management that respects the animals' hierarchy system and herd-feeling makes the **dehorning** of cattle unnecessary (Menke et al., 1999). Dehorning is a sign of inadequate husbandry, but it is also a distressing and painful experience for the animals (Taschke, 1995).

The **tie-stall** is an extremely uncomfortable [hard surface], painful [risk of inflammations of knees and hocks], frustrating [lying down is aversive, but there is a strong urge to rest in recumbence], and boring [restricted or no opportunity for social contact/interaction and foraging] housing environment (Krohn and Munksgaard, 1993; Redbo, 1993; Krohn, 1994; Haley et al., 2000) and, therefore, is not appropriate for cattle who are expected to yield research data that are not confounded by impaired well-being. The inadequacy of the tie-stall is reflected in the frequent occurrence of stereotypical activities [e.g., bar-biting, tongue-rolling], which disappear when the animals are transferred to loose housing or pasture (Redbo, 1992; Krohn, 1994). If circumstances require that a cow is temporarily tethered—e.g., venipuncture, remote sample collection via indwelling catheter—she should be tied by a halter and released as soon as the procedure is completed. A temporarily tied or single-housed animal must always be able to keep at least visual contact with other close-by members of the herd to buffer stress reactions. Under exceptional experimental circumstances lasting less than a day, a mirror may substitute for another conspecific (Piller et al., 1999).

- **Long-lasting affiliative relationships** exist not only among friends—who prefer each other as grazing, grooming, and resting partners (Reinhardt, 1980; Reinhardt and Reinhardt, 1981a; Reinhardt et al., 1986)—but also between mother and offspring. The bond between cow and calf is not affected by the weaning process—which occurs when the calf is approximately 10 months old (Reinhardt and Reinhardt, 1981b)—but lasts many years, leading to the development of tight-knit family subgroups within the herd (Reinhardt and Reinhardt, 1981a; Reinhardt et al., 1986; Figure 4).

In the research setting, distress has to be avoided to guarantee the scientific validity of research data. It is, therefore, not justifiable to subject the dam and the calf to the extremely disturbing situation created by premature **weaning.** Forced weaning distresses both the cow, who will show reduced reproductivity as a result of it (Reinhardt, 1982), and the calf, who will be prone to develop behavioral signs of frustration (Seo et al., 1998) and whose physiological ability to cope with stress will be

Figure 5. The grass field is the most appropriate living environment for cattle. Here two friends, *Nanette* and *Gilla*, keeping each other's company while grazing (cf., Figure 1).

Figure 6. Cattle must have access to shaded areas to forestall heat stress.

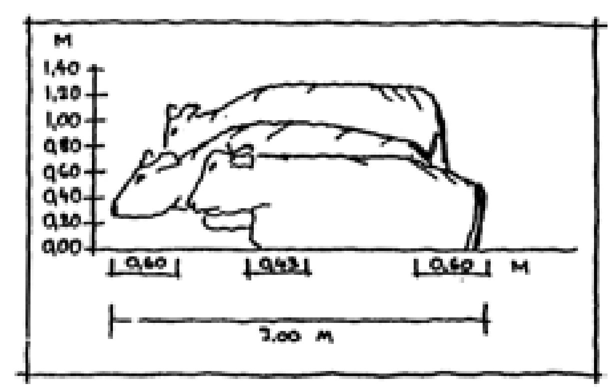

Figure 7. Cattle need considerable space in front of them to "swing" up into a standing position (drawing by H. Hoffmann).

impaired (Lay et al., 1992). If calves are allowed to stay with their mothers until the natural weaning process has occurred, they will not engage in compulsory substitute sucking, which often leads to health problems associated with the development of bezoars. It is not the substitute sucking that deserves the label "abnormal" (Loberg and Lidfors, 2001) but the human interference with a natural process (Reinhardt and Reinhardt, 1980a).

- Under natural conditions **cattle divide the day time into long periods of foraging** while moving considerable distances and long periods of chewing the cud while loafing or resting in a recumbent position (Reinhardt, 1980; Krohn and Munksgaard, 1997). When given the choice, cattle will spend almost all their time on a grass field rather than in a stable with deep bedding (Krohn et al., 1992). The urge to forage is so strong that well-fed cattle will push their way through a fenced area to get access to a meadow where they can graze (Trantham, 2000).

For cattle used in research, a well managed **pasture** is the most appropriate living environment (Krohn and Munksgaard, 1997; Figure 5). That's where they can graze ad libitum and that's where they can find suitable places to lie down comfortably and rest undisturbed in cattle-specific recumbent positions. The enhanced well-being of cattle on pasture is reflected by a high degree of herd synchrony and the absence of restlessness that typically occurs indoors as a result of spatial restriction (cf., O'Connell et al., 1989; Miller and Wood-Gush, 1991). Rotational grazing is the most suitable management system providing the animals adequate foraging opportunities, while fostering their health (Beetz, 1999).

Cattle seek out **shady places** during the hottest time of the day because they are very susceptible to heat stress (Kidd, 1993; Silanikove, 2000; Mitlöhner et al., 2001; Figure 6). If the pasture does not include trees or other shade-casting structures, a shademobile should be

Figure 8. Straw is a cattle-appropriate resting substrate and should be used whenever possible (©STS, photo by Hans-Peter Haering).

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Gerriet Trei is a Ph.D. student with Professor Fölsch. He works on feeding behavior, optimizing the composition of food for hens. He has collaborated with Detlef Fölsch in the projects "Alternative Housing Systems for Poultry in Hessia" and "Animal and Environmental Friendly Housing Systems for Poultry" of the German Ministry for Consumer Protection, Food and Agriculture.

Comfortable Quarters for Amphibians and Reptiles in Research Institutions

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There are approximately 6000 species of reptiles and 4000 species of amphibians. Some are completely aquatic, some rarely leave the trees, and some are burrowers. They are found in almost every habitat on the planet. At first glance, it would appear difficult to list criteria for laboratory housing for such a diverse group of animals. However, there are three general keys to successful housing:

1. a knowledge of the biology of the specific species including the basic, but essential needs of ectotherms;
2. an ability to replicate the most important features of the reptile's or amphibian's natural environment in the housing and care provided to the animals in the laboratory; and
3. caretakers who are able to recognize signs of discomfort, stress, and ill health in the particular species.

- Reptiles and amphibians are **ectotherms** [cold-blooded animals]. Unlike endotherms [warm-blooded animals], their body temperature is strictly dependent on the ambient environment.

  The advantage of ectothermy is that the resting metabolic rate and general energy requirements are less than those for mammals or birds of comparable size since no metabolic energy is spent on warming or cooling the body, and less energy is spent on searching for prey because less food is required to meet the body's low energy demands. The disadvantage of ectothermy, however, is that the ambient temperature determines the animal's metabolic processes and behavior. The animal must actively seek temperatures that will allow him or her to feed, digest food, hibernate, etc. Reptiles and amphibians literally "select" their body temperature by finding the appropriate thermal environment through basking, burrowing, hiding under logs or leaves, or entering water. For example, after a meal, snakes will move towards a heat source to aid digestion, and they will retreat to a cooler area following defecation.

  In many respects cold-blooded animals are more interactive with their environments than warm-blooded animals. At the same time, they tend to have greater problems adapting to changes in their species-typical environment (Warwick, 1987; cf. Wright, 1994). Therefore, the design of their artificial habitats demands special care if research-biasing stress and distress responses to species-inadequate environmental conditions are to be avoided. "Whether an observer maintains a high personal respect of 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, 1990a, p. 363).
- The knowledge of the thermal limits of a species is a basic condition for its proper care. Individual animals must be observed regularly and carefully to assure that their microhabitat suits their thermal requirements. If a reptile or amphibian spends all the time under or on the heat source, the ambient **temperature** is—obviously—too cool. If the animal stays as far away as possible from the heat source, the temperature is too warm.

  Temperature is best timer-controlled, taking natural temperature gradients [evening temperatures drop significantly in the desert for example] at the individual vivarium or tank level into account. Depending on the size of the enclosure, a gradient can be established by using either radiant heat from quartz heaters used to brood chicks, 25 to 250 watt incandescent light bulbs placed

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