

VARIABLES, REFINEMENT and  
ENVIRONMENTAL ENRICHMENT  
for *Rodents and Rabbits* kept in  
RESEARCH INSTITUTIONS



*Making Life Easier for  
Animals in Laboratories*

by Viktor and Annie Reinhardt  
Animal Welfare Institute

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Institute



We dedicate this book to the numerous  
animal caregivers, animal technicians and  
clinical veterinarians who have the courage to actively  
express compassion in their daily work with animals  
assigned to biomedical research and testing.

*Variables, Refinement and Environmental Enrichment for Rodents and Rabbits  
kept in Research Institutions: Making Life Easier for Animals in Laboratories*

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# 1. INTRODUCTION

Permanent confinement inevitably carries the risk of distress. Being forcibly restrained during potentially life threatening, painful and uncomfortable procedures also intrinsically carries the risk of stress. Both circumstances have traditionally been given too little or no attention in the husbandry practices of rodents and rabbits used in biomedical research. This is surprising, because distress and stress jeopardize not only the welfare of the research subject but also the validity of the scientific data.

A respected researcher conceded in the prestigious journal *Laboratory Animal Science*:

“Most investigators think only briefly about the care and handling of their animals and clearly have not made it an important consideration in their work” (Traystman, 1987, p 108).

This attitude is not conducive to changing the traditional husbandry practices of rodents and rabbits. Many animal caregivers and technicians are increasingly frustrated with the *status quo* and are looking for ways to make life easier for the animals in their charge.

This book offers some guidance. It summarizes and discusses refinement and environmental improvement techniques for rodents and rabbits kept in research facilities. The review of the literature focuses on data-supported published material and mentions descriptive and theoretical articles only if they have practical relevance. It does not include research pertaining to the handling of young animals (gentling) and its effect on their emotional development.

A total of 260 relevant articles published in 85 different journals have been reviewed. The species studied were:

- rats 100 articles (38 percent)
- mice 87 articles (33 percent)
- rabbits 35 articles (14 percent)
- guinea pigs 15 articles (6 percent)
- hamsters 15 articles (6 percent)
- gerbils 7 articles (3 percent)
- bank voles 1 article (<1 percent)

Of the 260 articles:

- 137 articles (53 percent) were published in European journals.
- 120 articles (46 percent) were published in US journals.
- 3 articles (1 percent) were published in journals of other countries.

The majority of articles were published in the following journals:

- *Laboratory Animals*, 31 articles
- *Contemporary Topics in Laboratory Animal Science*, 19 articles
- *Animal Technology (and Welfare)*, 15 articles
- *Animal Behaviour*, 14 articles
- *Physiology and Behavior*, 13 articles
- *Animal Welfare*, 13 articles
- *Applied Animal Behaviour Science*, 13 articles
- *Behaviour*, 10 articles

In order to make the text more succinct, some methodological details have been deliberately omitted. The age category and sex of the subjects tested, the number of test subjects, and the dimension of the primary enclosure are included in this review only if the author of a publication refers to this information in the discussion of the research findings and/or if this information is important to the interpretation of the data.

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## 2. VARIABLES AND REFINEMENT

Variables are uncontrolled factors that have the potential to influence research data.

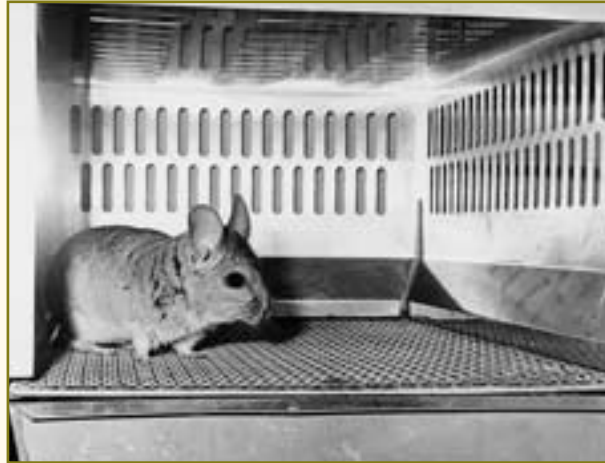
Refinement is the attempt to enhance animal welfare by reducing to an absolute minimum the amount of distress imposed (Russell and Birch, 1959) and control extraneous variables that may increase research data variability (Reinhardt and Reinhardt, 2002) and, hence, increase the number of research subjects needed to achieve statistically significant results (National Research Council, 1985; Home Office, 1989; Institute for Laboratory Animal Research, 1992).

### 2.1. Confinement in Barren Cages

#### 2.1.1. Variables

Being confined in a barren primary enclosure is probably the most serious stressor for animals, because it isolates them from their surroundings (Figure 1). For rodents and rabbits, empty space is biologically extremely frightening as it exposes them to the eyes of potential predators. But there is an absolute minimum empty space that the caged animals need, otherwise there is a risk that their physiological equilibrium will become disturbed.

Kuhnen (1998,1999) compared the fever response—a non-specific immune response—to the administration of *Salmonella typhosa* lipopolysaccharide in golden hamsters, who were individually housed either in small unfurnished cages (200 cm<sup>2</sup>) or in large unfurnished cages (1815 cm<sup>2</sup>). Subjects in the small cages showed a significantly reduced febrile response relative to those in the large cages. This was taken as an indicator that housing in too small cages induced chronic stress. Fullwood et al. (1998) examined trios of male C57BL/6 mice housed in barren cages that were very small (32 cm<sup>2</sup>/mouse), small (64 cm<sup>2</sup>/mouse), meeting official space recommendations (97 cm<sup>2</sup>/mouse) or relatively large (129 cm<sup>2</sup>/mouse). Mice in the very small cage had a significantly greater lymphocyte blastogenesis than all the other mice, who showed no significant differences in this particular stress parameter. Both adrenal weights and plasma corticosterone concentrations, however, increased significantly with reduced cage size.



**Figure 1. Being permanently confined in a barren cage without companionship is a distressing experience for rodents—here a chinchilla—and rabbits alike.**

Beyond a minimum required for freely engaging in species-typical postures and postural adjustments (e.g., outstretched lying posture, turning) and species-typical locomotion patterns (e.g., hopping, leaping, climbing) space has little value unless:

- it is furnished with structures/objects that entice the subject to explore and make use of them in a species-typical manner, or
- it is shared with one or several compatible conspecifics to allow for social contact and interaction.

Being confined in an unstructured primary enclosure is often associated with:

- a high level of stress, anxiety and fear
- maladaptive behaviors such as hair-pulling and -eating, bar/wire-gnawing and sham digging. (These seemingly senseless, often monotonously repeated activities are likely to reflect abnormal brain function (Garner et al., 2003). They are also referred to as behavioral pathologies (Erwin and Deni,

1979) and commonly interpreted as signs of distress (Institute for Laboratory Animal Research, 1992; Organisation for Economic Co-Operation and Development, 2000; Garner and Mason, 2002.)

- hyperaggressiveness
- suboptimal development of brain functions and alterations in brain neurochemistry
- poor recovery from experimentally induced brain injuries and worsening in the progression of neurological disorders

Numerous attempts have been made to ameliorate these problems by furnishing the animals' impoverished living quarters with species-appropriate structures, offering the animals objects to interact with in non-injurious ways and providing them with compatible companionship.

## 2.1.2. Refinement

### 2.1.2.1. Reduction of Stress, Distress, Fear and Anxiety

Stress is an effect produced by acute events, referred to as stressors, that induces an alteration in the subject's biologic equilibrium.

Distress is a subjective state that results from the inability to adapt to a chronic stressor or to a repeatedly occurring stressor. The inability to adapt is reflected in aversive gestures and behavioral pathologies and in significant physiological deviations from the undisturbed state.

Anxiety is a subjective state triggered by an *unknown* potential danger. For example, an animal who has repeatedly been exposed to a painful, life-threatening situation by various investigators might retreat to the back of his/her homecage whenever a person enters the room.

Fear is a subjective state triggered by a *known* danger. For example, an animal who has repeatedly been exposed to a life-threatening procedure by a certain investigator squeals and crouches in the back of his/her homecage when this investigator enters the room but not when another person enters.

Gardiner and Bennett (1978) measured the blood pressure of Wistar **rats** who were kept either alone in a



**Figure 2. Compatible social companions and species-appropriate environmental enrichment can buffer the stress resulting from permanent confinement in artificial living quarters.**

barren cage or as pairs in two interconnected barren cages. While all five single rats developed hypertension after five days, none of the five paired rats became hypertensive during this time period. This suggests that the presence of a companion mitigated the stress response (social buffer) to a rat-inadequate living environment.

Cambardella et al. (1994) noticed that individually caged Sprague-Dawley rats experienced more stress, as reflected in significantly higher concentrations of plasma corticosterone and prolactin, than subjects housed six per cage (Figure 2). Sharp et al. (2002, 2003) recorded the behavior and the telemetrically measured heart rate of Sprague-Dawley rats housed either alone or in groups of four same-sex partners in 930-cm<sup>2</sup> standard cages. In both sexes resting heart rates were lower in group-housed rats. Moreover, group-housed rats had significantly lower heart rates in response to disturbing husbandry procedures (e.g., cage change). Husbandry-induced arousal behaviors were less frequent and of shorter duration in group- than in single-housed rats.

Baldwin et al. (1995) kept Sprague-Dawley rats in 1200-cm<sup>2</sup> unfurnished cages alone or as a group of five. Basal corticosterone levels and lymphocyte percentages were significantly higher in single-housed rats than in group-housed rats, regardless of the fact that single rats had access to a relatively much larger, albeit unstructured, living space. Unlike space, social companionship seemingly buffered the stress attendant with confinement. The lower stress level in group-housed rats may account

for the observation that the incidence of pituitary tumors is conspicuously lower in animals housed in small groups than in those housed alone (Nyska et al., 1998).

Brown and Grunberg (1995) showed in female Wistar rats that the high corticosterone level associated with small living quarters is significantly reduced by the presence of other females, even if this implies crowding (four animals in a 405-cm<sup>2</sup> cage). This effect could not be verified in males who, unlike females, are more stressed (higher corticosterone concentration) under crowded than under single-housing conditions. Social deprivation, therefore, is a particularly potent stressor for female rats.

Shaw and Gallagher (1984) noticed in Sprague-Dawley rats that animals housed in same-sex groups of five in 1820-cm<sup>2</sup> cages had a significantly longer survival rate under undisturbed conditions than individuals kept alone in 600-cm<sup>2</sup> cages. Male rats were prone to develop foot lesions resulting from the wire mesh floors. The incidence of such lesions was markedly lower in group-housed than in singly housed rats, presumably because of the potential for more exercise in the large group-cages.

Conger (1957) placed Hooded Norway rats in a distressing conflict situation and noted that pair-housed animals were protected from stress, showing a greater resistance to the development of gastric ulcers than singly housed rats. Patterson-Kane et al. (1999) recorded significantly higher latencies to emerge from a box and explore a novel environment in Hooded Norway rats who were housed alone compared to those housed in pairs. From this it was inferred that the pair-housed rats were less fearful.

Heath (1999) observed rats of unspecified strain who were caged either alone or with another rat. While singly caged individuals spent most of their time in the back of the cage, pairs spent most of their time in the front of the cage, suggesting that they experienced less fear in the presence of the observer.

Davitz and Mason (1955) subjected Wistar rats to an open-field situation and noted that the behavioral fear response of the subjects was significantly less intense when they were tested together with another rat versus alone. Latané (1968) and Taylor (1981) extended these studies and demonstrated that:

- a. The fear-buffering effect—as seen by less frequent defecation and freezing in a fear-provoking situation—is dependent on the other

rat being conscious, not anesthetized.

- b. A strange rat allays fear as effectively as a familiar rat.
- c. Inanimate enrichment objects have no moderating effect on the subject's fear response.

Green et al. (2002) implanted male Sprague-Dawley rats with jugular catheters that allowed the animals to self-administer the psychoactive drug amphetamine. Animals living alone in barren 410-cm<sup>2</sup> standard cages self-administered significantly more of the drug than animals who shared a 7200-cm<sup>2</sup> cage with nine other males. The combined effect of companionship and more space may have decreased the urge for the presumably hedonic effect of the drug in an environment that was more species-appropriate than the boring single-cage.

Sharp et al. (2003) monitored telemetrically heart rate and blood pressure in undisturbed male Sprague-Dawley rats who were housed with three other rats in small (920 cm<sup>2</sup>) unfurnished cages or in large (1250 cm<sup>2</sup>) unfurnished cages. The parameters measured did not differ between the two test groups, showing that additional *unstructured* space is unlikely to reduce potential stress resulting from confinement. Hirsjärvi (1994), however, found in pairs of male Wistar rats that the subjects were less fearful—as measured by shortened latency to rear when the cage top is removed—when the floor area of their standard cages (800 cm<sup>2</sup>) was enlarged by 16 percent (to 930 cm<sup>2</sup>) and the height of their cages (15 cm) increased by 33 percent (to 20 cm).

Belz et al. (2003) examined the effects of environmental enrichment on stress-sensitive hormones in vein-cannulated individually caged Sprague-Dawley rats. The cages of control animals were barren while those of the test animals were enriched with rubber toys and squares of compressed cotton fiber (nestlets), two items that were used for gnawing and shredding. Rats with enrichment had significantly lower plasma adrenocorticotropin and corticosterone concentrations than those with no enrichment. Foulkes (2004) kept Wistar rats in single-cages that were barren or enriched with a polyvinyl chloride (PVC) tube for hiding. Chromodacryorrhoea, a rat-specific stress indicator (Mason et al., 2004), was significantly less pronounced in the enriched as compared with the non-enriched rats, suggesting that the animals were less stressed when they had access to a tube.

Sharp et al. (2005) measured heart rate and blood pressure of spontaneously hypertensive (SH) and Sprague-Dawley rats housed individually in 930-cm<sup>2</sup> cages that were barren or enriched with a simulated burrow, a feeding enrichment gadget, and a shredding-and-nesting item. Blood pressure was not affected by enrichment. Heart rate, however, was significantly lower in SH rats who lived in enriched vs. barren cages. This effect could not be confirmed in Sprague-Dawley rats.

Eskola and Kaliste-Korhonen (1998) furnished the home cages of trio-housed Wistar rats each with three blocks of aspen wood. The animals gnawed these blocks into small pieces and did not lose interest in them over time. They ate less, as reflected in significantly lower weight gains, were more active, and exhibited the alert-posture less frequently when tested in an unfamiliar open-field area than control animals kept in barren cages of the same size. It was concluded that access to the gnawing blocks made the rats less timid.

Klein et al. (1994) exposed isosexually group-housed Sprague-Dawley rats—six animals per group—to cat urine. Animals living in cages that were enriched with eight daily changed, unspecified toys showed significantly fewer freeze-and-concealment responses to this natural predator stressor than animals in barren cages of the same size. This was evident in both sexes and taken as an indicator that toys mitigated the stress response and lowered the level of anxiety in the subjects.

Mlynarik et al. (2004) compared the corticosterone response induced by repeated injections of *Escherichia coli* lipopolysaccharide in Wistar rats, who were kept either in groups of three in small, barren cages (1200 cm<sup>2</sup>) or in groups of ten in large cages (5000 cm<sup>2</sup>) enriched with several platforms, swings, tunnels, glass jars, branches, running wheels, cardboard boxes and a water pool. Lipopolysaccharide injections resulted in plasma and adrenal corticosterone levels that were significantly increased in the first but not in the second group of animals. Living in a larger and more complex environment may have made the second group of animals more resistant to the immune challenge.

Ader et al. (1991) assessed emotionality in non-obese diabetic (NOD) mice who were housed either alone or in groups of five or eight same-sex animals in barren standard cages. Individually caged mice of both sexes exhibited significantly more fear and anxiety—as determined by resistance to being picked up, vocalizing, struggling,

spontaneous urination and defecation—than mice caged in the company of other mice.

Einstein et al. (2000) used a telemetry system to monitor the heart rate of male BALB/c mice over an 18-day period. The animals were housed alone or as trios in unfurnished cages. Throughout the experimental period, individually caged mice had significantly higher heart rates than the group-housed mice, suggesting that they were more distressed. Späni et al. (2003) confirmed these findings in male outbred mice who were kept alone or as pairs with ovariectomized females in same-sized cages furnished with hay and paper towels. Single mice had significantly higher heart rates than their nine pair-housed counterparts. This is in line with Herreid and Schlenker's (1980) observation of male RR mice who had significantly higher metabolic and heart rates when they were kept alone than when they shared a cage with another mouse. Späni et al. (2003) also noticed striking differences in the subjects' sleep patterns, with single-housed mice showing more frequent, short phases of sleeping than pair-housed mice.

That the welfare of singly housed mice is impaired relative to those living with companions has also been emphasized by Andervont (1944), because C3H mice housed in groups of eight were less susceptible to developing mammary tumors than mice housed alone.

Chamove (1989) kept groups of six CLFP mice in same-sized cages that were either unstructured or furnished with several vertical partitions with passage holes, structuring the cage floor into a complex burrow-like system. Animals from the burrow-cage were less inclined to escape when the cage top was removed, and they deposited significantly fewer fecal boluses when tested in an open field, than control subjects from the unstructured cage. This was taken as a sign that the provision of vertical cage dividers reduced the stress associated with confinement and made the animals less fearful. Van Loo et al. (2004) measured significantly decreased urinary corticosterone concentrations in BALB/c mice kept in trios, when their cages were enriched with paper tissues that allowed individuals to break visual contact with each other and hide from disturbing environmental influences.

Kingston and Hoffman-Goetz (1996) noticed in C57BL/6 mice living alone or in groups of eight in barren cages that enrichment in the form of a running wheel, nesting material, small bottles, and tubes significantly buffered the animals' immunosuppression at times of stress. Benaroya-Milshtein et al. (2004) demonstrated in single- and group-

caged CrH/eB mice that the provision of ladders, tunnels and a running wheel mitigated immune responses to acute stress and significantly reduced anxiety- and fear-related behavioral responses in an unfamiliar environment.

Manosevitz (1970) tested random-bred mice in an empty arena. Animals raised with their littermates in large cages (5350 cm<sup>2</sup>) enriched with a variety of structures for climbing and hiding defecated less often than animals raised in small, barren standard cages (500 cm<sup>2</sup>). Similar findings were reported for outbred mice as well for BALB/c and C57BL/6 mice (Scharmann, 1994; Chapillon et al., 1999), suggesting that anxiety and fear can be buffered by inanimate environmental enrichment.

Sherwin and Olsson (2004) offered C57BL/6 mice free access to a solution of a psychoactive anxiolytic (midazolam) and compared trios housed in unfurnished 540-cm<sup>2</sup> cages with trios kept in more spacious 1090-cm<sup>2</sup> cages that were enriched with a nest box, a running wheel, cardboard tubes and nesting material. The enriched mice drank significantly less of the midazolam solution than the non-enriched mice, suggesting that more space along with enrichment made the animals less anxious. Van de Weerd et al. (2002) came to a similar conclusion when assessing fear-related behaviors of RIVM mice kept in groups of eight in small barren cages (375 cm<sup>2</sup>) or in large cages (840 m<sup>2</sup>) furnished with gnawing blocks, climbing structures, a nest box and wood-wool.

Roy et al. (2001) assessed plasma corticosterone concentrations of BALB/c mice who had been exposed to cat feces in their familiar home environment. The subjects were raised with their littermates either in large (1300 cm<sup>2</sup>) cages each containing a running wheel and unspecified enrichment objects or in small (260 cm<sup>2</sup>) unfurnished standard cages. Mice from large enriched cages showed a significantly lower corticosterone stress response to the natural stressor than mice from small, unenriched cages.

Manosevitz and Pryor (1975) demonstrated in C57BL/6 mice that space alone can have a stress-buffering effect. The test animals were all kept individually in barren cages that were either small (440 cm<sup>2</sup>) or large (5350 cm<sup>2</sup>). When exposed to an unprotected open field, mice from large cages demonstrated significantly fewer signs of anxiety and fear (i.e., less defecation and more exploration) than the mice from small cages.

McMahon et al. (2005) determined that breeding trios of C57BL/6 mice had a much higher birth rate (9.6 pups/female) in presumably unfurnished, relatively large 860

cm<sup>2</sup> cages than in small 375 cm<sup>2</sup> standard cages (7.2 pups/female). Keeping the mice in larger cages also provided a better microenvironment as measured in lower ammonia levels (17 ppm vs. 24 ppm).

### 2.1.2.2. Alleviation of Maladaptive Behaviors

Maladaptive behaviors reflect the inability of a subject to adapt to species-inappropriate living conditions. Maladaptive behaviors are generally referred to as *abnormal* behaviors. This misleading term is avoided here, since it is the species-inappropriate conditions under which the subject is forced to exist that are really abnormal, not the subject's attempts to adapt to them.

Baenninger (1967) compared the behavior of singly caged **rats** of unspecified strain with that of rats kept in groups of six. Both categories of animals were housed in 1740-cm<sup>2</sup> cages and their behavior was monitored from the day of weaning (21 days old) until the age of 92 days. Stereotypic pawing and tail-manipulating developed under both housing conditions, but their occurrence was significantly lower in the group-housed rats.

Callard et al. (2000) videotaped isosexually pair-housed roof rats in 1900-cm<sup>2</sup> cages that were either empty or furnished with 13 x 25 x 20-cm wooden shelters. The rats engaged in stereotypic backflipping under both conditions, but the incidence and frequency of this behavior were significantly lower when they had a shelter.

Wrightson and Dickson (1999) and Van Berkum (2000) controlled the risk of excessive eating resulting from boredom in group-housed Sprague-Dawley rats by inserting metal plates over the food hoppers, so that only a small section of the original food access area remained available. The animals worked harder for their food, which made them burn more calories and allowed them to eat throughout the day. After an eight-month test period they were slim, healthy and significantly lighter than control rats with unrestricted food hoppers (Wrightson and Dickson, 1999).

DeLuca (1997) assessed alopecia resulting from partner-directed hair-pulling (“barbering”) in groups of ten **mice** of unspecified strain. The animals were kept in cages of unspecified size that were either barren or furnished with nest boxes and various commercial, regularly replaced toys. The incidence of hair loss due to barbering was about 60 percent in unenriched mice, but only 23 percent in enriched mice.

Leach et al. (2000) tested a custom-made cage insert consisting of two raised platforms and a shelter. Videorecordings of pair-housed BALB/c mice revealed that the animals showed significantly less bar-gnawing with the insert. Würbel et al. (1998) made a similar observation in pairs of male ICR mice. The provision of cover, in the form of a cardboard tube, in an otherwise barren, hence potentially fear-inducing cage significantly reduced, but did not eradicate stereotypic wire-gnawing.

Powell et al. (2000) studied the behavior of deer mice (*Peromyscus maniculatus bairdii*) reared (a) in same-sex pairs or trios in barren standard cages or (b) in a same-sex group of 16 animals in a cage that was more than 500 times larger and furnished with wire mesh cylinders, PVC tubes, and dividers. Stereotypic jumping, backward somersaulting and patterned running dominated the behavioral repertoire of the animals in the small barren cages (Powell et al. 1999). Given the constraint of confinement, these stereotypies developed even in the very large and complex enclosure, but their occurrence was significantly lower than in the small, unfurnished cages (Powell et al., 1999, 2000).

Wiedenmayer (1997) developed an artificial burrow system for **gerbils**, who are notorious for their stereotypic digging. The burrow consists of an opaque nestbox with an angled access tube. Providing the cages of gerbil families with this burrow system almost entirely prevented the development of stereotypic digging in young animals (Wiedenmayer, 1997) and reduced its occurrence significantly in adults (Waiblinger and König, 2004).

McClure and Thomson (1992) noticed that golden **hamsters** kept individually in suspended wire cages developed bizarre aggressive behavior. They spent much time growling, hissing, posturing aggressively toward humans, destroying the rubber stoppers of their water bottles and attacking any objects introduced into the cage. Many animals developed inappetence which progressed to anorexia, depression and unresponsiveness. When the hamsters were given cotton nestlets their appetite and responsiveness improved, but their aggressive behavior remained unchanged. When the nestlets were replaced by a PVC pipe section (13 cm long, 5.5-cm diameter) functioning as a burrow, the aggressive behavior diminished within three days and was no longer discernible after 14 days.

Krohn et al. (1999) adjusted the routine feeding time of singly caged Ssc:CPH **rabbits** from four hours after daybreak to two hours before nightfall, which is about the natural time of day when wild rabbits would forage and eat.

This simple change in feeding time resulted in a significant reduction of stereotypic activities from approximately 4.5 to 0.5 percent of the time during the night (dark phase of the artificial light cycle). The rabbits displayed stereotypies during the day (light phase of the artificial light cycle) only 0.5 percent of the time. The change in the feeding schedule did not diminish the occurrence of stereotypic behavior even further.

Brummer (1975) noticed hair-pulling and -eating as a common behavioral problem in a colony of New Zealand White (NZW) rabbits housed in barren cages. When 14 does were provided with straw, they stopped pulling their hair, and the development of this behavioral disorder was prevented in all their offspring. Unfortunately, this clinical study is not supported by data.

Potter and Borkowski (1998) diagnosed three NZW rabbits, housed singly in barren cages, with psychogenic polydipsia (over-drinking). Placing hay and unspecified toys into the cages resulted in a conspicuous decrease in the manifestation of this maladaptive behavior in all three cases.

Lidfors (1997) offered NZW rabbits housed individually in barren cages (a) a plastic bottle filled with 20 g hay twice a day or (b) a regularly replaced aspen gnawing stick. It was a relatively lengthy task for the rabbits to pull hay out of the bottle, but this opportunity to spend extra time foraging correlated with a significant decrease in the occurrence of excessive fur-licking, sham chewing and bar-gnawing. The gnawing stick was chosen rarely and did not alter the rabbits' engagement in these behavior patterns. No changes in maladaptive behaviors were registered when the animals were given access to a wooden or plastic box. The rabbits hardly ever made use of such a potential shelter (Lidfors, 1997; Berthelsen and Hansen, 1999, Hansen and Berthelsen, 2000). Berthelsen and Hansen (1999) daily replenished hay on top of the barren cages of individually housed NZW x French Lop rabbits. This decreased, albeit insignificantly, the percentage of time that the animals spent bar-gnawing from approximately nine to four percent.

Moore and Beeston (1999) developed an unconventional cage for singly housed rabbits. Its front protrudes, giving the subjects improved vision out of the cage; a shelf in the back of the cage offers a comfortable resting site and a covered retreat area underneath; and a hay rack serves as a feeding enrichment device. The benefit of this cage design for the animals has yet to be demonstrated.

Edgar (2004) furnished the cages of individually



Joanne Edgar, Edinburgh University, UK

**Figure 3. A mirror does not seem to attract rabbits, but it distracts them sufficiently to reduce the incidence of stereotypical bar-biting.**

housed NZW rabbits with an interior mirror (Figure 3). Seven-day videorecordings revealed that this reduced the time spent engaged in bar-gnawing from 1.2 to 0.2 percent in males and from 0.3 to 0.2 percent in females.

Gunn-Dore (1999) provisioned singly caged NZW rabbits with brass wire balls (5-cm diameter) that the animals could chew and push around. During a three-week test period female and male rabbits progressively lost interest in these toys, but spent significantly less time engaged in stereotypic hair-chewing and bar-gnawing.

Stauffacher (2000) compared the behavior of female Belgian Hare rabbits who were kept alone or with another female. Wire-gnawing and wire-licking occurred under both housing conditions, but the incidence of these behaviors was significantly lower in the pair- than in the single-housed animals. Chu et al. (2004) raised female NZW rabbits in barren standard cages or as pairs in double-size cages. The animals showed bar-gnawing in both housing conditions, but while the incidence of this behavioral pathology remained relatively low in paired rabbits, it showed a significant and progressive increase in single rabbits. Kalagassy et al. (1999) confirmed in Flemish Giant x Giant Chinchilla rabbits that stereotypic behaviors occur only in animals who are housed alone, but not in those housed with a companion.

Gunn and Morton (1994) observed female NZW

rabbits who were caged individually in small barren quarters or as a group of nine in a large, enriched enclosure. While each of the singly caged does showed wire-gnawing about 13 percent of the time, stereotypic behaviors were not observed in the socially housed does, who had access to straw and cardboard boxes. It is not clear which of these environmental improvements accounted for the absence of maladaptive behaviors.

Held et al. (2001) studied groups of four adult female NZW rabbits in pens furnished with cardboard boxes for hiding and gnawing and raised platforms for rearing up, jumping up, and resting in an elevated position. In addition, the animals had access to straw and hay for burrowing and foraging. The subjects were watched for a total of 189 hours over a period of 2.5 years, but no stereotypic behaviors were ever observed. The absence of stereotypic behavior in group-housed does has been emphasized by Loeffler et al. (1991). Krohn et al. (1999), however, *did* observe some stereotypies at night, but not during the day. This suggests that group-housed rabbits may well engage in stereotypic behaviors during the night when they are active, but nobody is observing them.

### 2.1.2.3. Reduction of Aggression

Gwinn et al. (1999) provisioned groups of five male Swiss nude **mice** with nestlets and noticed a 31 percent decrease in fight injuries compared to controls. Armstrong et al. (1998) compared aggression among groups of eight male BALB/c mice in same-sized cages that either had standard bedding or were enriched with natural cornhusk covering the cage floor to a depth of 2.5 cm. Mice maintained on the cornhusk had significantly fewer wounds than those on standard bedding, probably because the husks encouraged burrowing and nesting and hence the option of moving out of the immediate vicinity of cagemates.

Arnold and Westbrook (1997/1998) observed same-sex groups of four golden **hamsters** in cages that were either barren or furnished with a T-shaped PVC pipe or with a pint-sized clear glass jar. Hamsters with access to these objects displayed only 34 and 22 percent, respectively, of the aggression displayed by hamsters kept in barren cages.

Arnold and Gillaspay (1994) kept female and male golden hamsters alone or as a group of four same-sex siblings in barren cages. Socially housed subjects were relatively docile. Their number of biting attempts against handling personnel was about a third of that shown by individually housed hamsters.

### 2.1.2.4. Enhanced Development of Species-Typical Brain Functions

Tagney (1973) studied the sleep patterns of **rats** in relation to their housing conditions. Animals kept in groups of six in cages equipped with a variety of enrichment objects and structures spent significantly more total time asleep—as seen in both slow wave sleep (SWS) and rapid eye movement (REM) sleeping times—than their littermates kept alone in barren cages. Evidence suggests that sleep is the time during which the brain synthesizes macromolecules required for its restoration after mental activity. The extra sleep of the rats living in a mentally stimulating environment presumably served this function. This assumption is supported by the findings of Diamond et al. (1964, 1972) and Diamond (1988, 2001), who demonstrated that the thickness of the cerebral cortex was lowest in rats kept alone. The provision of more space and access to running wheels, ladders and small mazes did *not* promote cortical thickness as long as the rat was kept alone. Cortical thickness increased significantly when a rat was allowed to share a cage with other rats. When enrichment was then provided in the context of social housing, cortical thickness increased even further. The positive effect of social partners and the combination of companionship plus enrichment was significant in subjects of all age classes. These findings indicate that living with other rats was the key factor for enhanced brain function and that companionship facilitated the brain-stimulating effect of environmental enrichment.

Valzelli (1973) reported that **mice** housed in groups of eight quickly learned to press lightly with their tongues on a lever in order to receive water from a drinking device. By contrast, 32 percent of an unspecified number of mice housed singly seemed unable to learn how to operate the automatic water distributor and died due to dehydration within one week. (It is difficult to understand why the author did not intervene in time to spare the animals the agony of death resulting from dehydration.)

Henderson (1970) raised groups of four mice in small, barren standard cages, and five-times larger cages that either were also barren, or enriched with a variety of objects for climbing, exploring, hiding and gnawing. The brain weight of subjects raised in a barren environment was not affected by the size of the cage, but it was significantly increased in subjects raised in enriched cages. Apparently, environmental enrichment was more important to brain development than additional unstructured space.

### 2.1.2.5. Recovery from Brain Injuries and Neurodegenerative Processes

Passineau et al. (2001) compared the recovery from traumatic brain injury in **rats** who were housed (a) alone in small, barren standard cages or (b) in groups of 14 animals in a large cage furnished with a running wheel, tunnels, a hammock, branches and a variety of toys. The combined effect of companionship, enrichment, and more space was reflected in significantly higher preservation of brain tissue integrity, along with significant attenuation of cognitive deficits. The enhanced recovery of cognitive function after brain injury in a social, more spacious, and more complex environment relative to the barren single-cage environment confirms the findings by Hamm et al. (1996). Farrell et al. (2001) made similar observations in gerbils kept in groups of five in large cages containing tubes, shelves, a running wheel and a wooden log versus alone in small barren cages.

It can be hypothesized that a more species-appropriate, complex environment stimulates mechanisms restoring function after brain damage (Ohlsson and Johansson, 1995; Mattsson et al., 1997; Belayev et al., 2003). Risedal et al. (2002) noticed in rats with experimental brain infarction that subjects recovered from surgery—as measured in motor function—significantly better when they shared a barren cage with other rats than when they were kept alone but had access to a running wheel. Johansson (1996) concluded from similar findings that companionship is more important to the recovery process than inanimate enrichment.

Ickes et al. (2000), Van Dellen et al. (2000), Hockly et al. (2002) and Spires et al. (2004) were able to slow neurodegenerative processes in group-housed transgenic **mice** by enriching the animals' cages with cardboard tubes for hiding and gnawing.

## 2.2. Cage Cleaning

### 2.2.1. Variables

Cage cleaning is usually a rather disturbing husbandry routine for rodents. This procedure can be associated with a significant:

- increase in activity and defecation in singly caged male rats (Saibaba et al., 1996)
- increase in blood pressure, heart rate and restlessness in male and female, single- and group-caged rats (Duke et al., 2001a; Duke et

- al., 2001b; Sharp et al., 2002, 2003)
- increase in core temperature and heart rate in male and female singly caged hamsters (Gattermann and Weinandy, 1996)
  - depression of a non-specific immune response in singly caged male hamsters (Kuhnen, 1999)
  - increase in overt aggression among group-caged male mice (Gray and Hurst, 1995)

### 2.2.2. Refinement

Gray and Hurst (1995) observed that aggression among the five members of group-housed CFLP mice escalated whenever the animals were briefly removed and subsequently placed back in their own dirty cages. The aggression eliciting effect of their own odor cues was replicated when the animals' home cage was not completely cleaned and deodorized but the soiled sawdust merely replaced with fresh sawdust. When the cage cleaning process involved the replacement of the soiled home cage with a new cage and fresh sawdust, inter-male aggression was substantially reduced, but not eliminated (Hurst, 1990).

Ambrose and Morton (2000) videotaped groups of five and six male BALB/c mice for one hour immediately after their cages were cleaned. The mice had their cages changed twice a week and were removed during this process from the soiled cage directly into a new cage.

- a. The incidence of inter-male aggression was significantly reduced when the new cage was provisioned with one cardboard box, one cardboard tube, and one wooden gnawing block.
- b. This aggression-buffering effect was nullified during the next cage cleaning when the three, in the meantime scent-marked, objects were transferred into the new cage.
- c. When the soiled objects, however, were replaced with identical new, scent-free objects, aggression was once again significantly decreased.

A significant reduction in aggression was also achieved by furnishing each cage with a glass jar that was thoroughly cleaned and deodorized as part of the cage cleaning process.

These findings confirm the aggression-inducing effect of familiar, territorial scent marks deposited on objects

(Jones and Nowell, 1973, 1975; Mugford, 1973; Gray and Hurst, 1995). They also demonstrate that the provision of species-appropriate enrichment—such as objects for seeking shelter (box, tube, bottle) or objects for gnawing (wood block)—can reduce the incidence of inter-male aggression and the associated risk of social distress and serious wounding, as long as they do not carry familiar odor cues. It should be noted that structural additions to the cage that do not provide escape routes/options can *increase* rather than decrease agonistic interactions among male mice right after cage cleaning (Van Loo et al., 2002).

Van Loo et al. (2000, 2004) videotaped trios of male BALB/c and CD-1 mice for one hour after their cages were cleaned. When the mice were transferred along with some of their soiled *nesting* material into clean cages, overt aggression was significantly reduced, and the animals' urine corticosterone concentrations were significantly lower compared with mice without nesting material. One may infer from this study and those of Gray and Hurst (1995) and Ambrose and Morton (2000) that specific scent marks deposited on the ground or on objects trigger territorial aggression while odor cues adhering to nesting material fail to induce, or perhaps even mitigate, aggression in male mice.

Armstrong et al. (1998) did not add enrichment objects but provisioned the weekly exchanged cage of eight male BALB/c mice with a 2.5-cm layer of fresh cornhusk. Observations carried out four days after each cage change revealed that the animals had significantly fewer wounds resulting from aggression than control animals, presumably because the mice could avoid conflicts relatively easily by breaking visual contact with each other in the husks. Van Loo et al. (2002) found in a subsequent study with trios of male BALB/c mice that agonistic interactions were significantly reduced immediately after cage cleaning when odor-free paper tissues—presumably along with fresh sawdust bedding—were added to the fresh cage.

Duke et al. (2001a) moved single male Sprague-Dawley rats to clean cages that contained new substrate plus a small quantity of the soiled bedding material from their previous cages. This failed to have a calming effect, and the subjects still showed significant cardiovascular (increased heart rate and blood pressure) and behavioral (arousal) responses. Repeated cage changing did not produce any lessening of these stress responses, suggesting that the rats could not adapt to this standard husbandry procedure.

## 2.3. Transfer to an Unfamiliar Location and Separation from Cagemates

### 2.3.1. Variables

Being removed from the home environment and transferred to an unfamiliar location is a very disturbing experience for captive animals, just as it is for humans. It has been documented in numerous reports that rats and mice experience significant changes in the resting values of physiological parameters when they are moved in their home cage to a different location (Friedman and Ader, 1967; Brown and Martin, 1974; Euker et al., 1975; Pfister and King, 1976; Kvetnansky et al., 1978; Gärtner et al., 1980; York and Regan, 1982; Damon et al., 1986; Ursin and Murison, 1986; Cabib et al., 1990; Drozdowicz et al., 1990; Tuli et al., 1995a; Barrett and Stockham, 1996; Tabata et al., 1998; Sharp et al., 2003). Surprisingly, such reports are missing for other rodents and rabbits, who are also likely to be stressed when they are transferred to different living quarters.

Being separated from familiar companions is distressing for any social animal, including rodents and rabbits. This experience is typically accompanied by behavioral fear responses such as freezing, reduced drinking and eating and associated loss of body weight, sustained increase in heart rate and blood pressure, and altered hypothalamic-pituitary-adrenal function and electroencephalic activity (Hadley, 1927; Fenske, 1990; Ehlers et al., 1993; Lawson and Churchill, 2000).

### 2.3.2. Refinement

Dobráková and Jurčovicová (1984) tried to habituate male Wistar **rats** caged in groups of four to being transferred in their home cage to another room, left there for a few minutes, and returned to the original location. This was repeated once every day for a period of 15 days. The animals were not able to adapt to this common procedure but showed significant increases in plasma corticosterone and prolactin which were not lower on day 15 than on day one.

Fenske (1992) was able to eliminate the typical freezing response to separation in group-housed male **guinea pigs** by confining the experimental subject in a small test cage that was placed in the familiar large home cage. This simple procedural adjustment allowed the subject to maintain uninterrupted auditory and olfactory contact with his cagemates.

## 2.4. Restraint

### 2.4.1. Variables

Being involuntarily handled and forcefully restrained by the human “predator” is a powerful stressor for rodents and rabbits. It jeopardizes not only their well-being but also the scientific validity of data collected from them (Balcombe et al., 2004). Späni et al. (2003) and Kramer et al. (2004) studied mice and showed that merely entering an animal room without touching a cage can be sufficiently alarming to trigger significant endocrine and cardiovascular stress responses that bear the risk of affecting subsequently collected stress-sensitive parameters, even *before* the actual experimental procedure is done with one of the research subjects of that room. Kramer et al. (2004) noticed that in individually caged C57BL/6 mice, males do not adapt to this everyday event. Subjects showed significant heart rate elevations and significant body temperature increases in response to personnel entering their room at 9:30 a.m., even after repetitions of this disturbance on 12 consecutive days.

### 2.4.2. Refinement

#### 2.4.2.1. Habituating to Procedures

Dobráková and Jurčovicová (1984) caught group-housed Wistar **rats** and handled each animal daily for one minute over a 15-day study period. Subjects showed elevated plasma corticosterone concentrations, but these were significantly lower on day 15 than on day one, indicating that the animals had adapted.

Sharp et al. (2005) recorded the heart rate and blood pressure of singly caged Sprague-Dawley rats who were given a subcutaneous injection once a day on four consecutive days. Injection resulted in a significant increase of both cardiovascular parameters and was accompanied by agitated movement. These responses did not change over the course of the four days, suggesting that the animals were not able to adapt to this common procedure within the given time frame.

Tuli et al. (1995b) demonstrated that singly caged BALB/c **mice** did not adapt to conventional restraint in perspex tubes. Even after 21 daily one-hour restraint sessions, elevated plasma corticosterone concentrations did not differ from those of subjects who were restrained only one time.

### 2.4.2.2. Training to Cooperate during Procedures

Gastric intubation for oral drug administration is probably the most distressing procedure that rodents and rabbits are subjected to routinely (Bonnichsen et al., 2005). The animal is usually exposed to two or three humans who apply forceful restraint/immobilization during an extremely uncomfortable, life-threatening, often injurious and sometimes even deadly procedure (Murphy et al., 2001; Murphy, 2001).

Huang-Brown and Guhad (2002) trained 57 Wistar **rats** living in trios to cooperate during daily oral administration of indomethacin and celecoxib, two anti-inflammatory drugs. An amount of medication equal to ten doses was mixed into approximately 500 mg of softened chocolate, and then divided into ten aliquots. The subjects were first allowed to develop a taste for pure chocolate by carefully placing a pellet into their mouth using a 14-gauge gavage needle. They were handled gently to avoid association of chocolate with aversive stimuli. After eight days of training, only five percent (3/57) of the animals failed to cooperate, while 95 percent (54/57) of them displayed “eager anticipation” of the decoy whenever the cage door was opened. The rats’ cooperative response did not change when the chocolate pellets contained the test drugs: they took and swallowed them without hesitation. This refined gavage method provided consistent, reliable, easy and accurate dosing. There was no need for keeping the animals individually. Housing them in small groups did not interfere with this new treatment technique.

Marr et al. (1993) developed a simple training technique to gain the cooperation of ten NZW **rabbits** for voluntary oral administration of the test drug tosufloxacin. For five days, the animals were offered sucrose water daily from a syringe. The tip of the syringe was coated with sucrose granules. Most rabbits spontaneously approached the syringe when it was inserted through the bars of the cage, tasted the tip of it and/or drank the fluid. Rabbits who did not initially seek the syringe usually did so with minimum encouragement. These training sessions were repeated three times a day, for a total of 15 minutes per session, until all animals swallowed the sucrose solution content of the syringe. The antibiotic solution was then substituted for the sucrose, while the tip of the syringe remained coated with sucrose granules for each subsequent daily administration of the drug. Within two days, eight of the ten rabbits were seeking the syringe when research staff entered the room.

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 solution” (Marr et al., 1993, p 48).

Needless to say, these rabbits did not experience any fear, distress or undue discomfort during this refined oral administration procedure.

There are no reports of training attempts to gain the cooperation of rodents or rabbits during injection or blood collection in order to reduce the stress reactions of the subjects resulting from enforced handling and restraint (Krulich et al., 1974; Moynihan et al., 1990; Brockway et al. 1993; Tuli et al., 1995c; Tabata et al., 1998).

### 2.4.2.3. Stress Buffer

The restraint stress experienced by the handled subject can be buffered under certain circumstances by the presence of one or several compatible conspecifics (social buffer) and by appropriate environmental modifications.

Ader and Friedman (1964) recorded the behavioral responses of Sprague-Dawley **rats** to being gently picked up by a person. Animals kept alone were more fearful of the handling person and showed significantly more alarm vocalization and resistance to being picked up than animals who shared a cage with five other rats. It was considerably more difficult to handle rats who were caged alone than those caged with other rats. Giralt and Armario (1989) found in Sprague-Dawley rats that their stress response to acute immobilization—as measured by the increase in plasma corticosterone concentration—was significantly greater when they were housed alone than when they lived in groups of four.

Sharp et al. (2002, 2003) worked with Sprague-Dawley rats bearing telemetry transmitters. Each subject was tested in single-housing and group-housing (four same-sex animals) conditions, and his/her heart rate monitored both during and after a subcutaneous injection. For this procedure, the cage was placed on a workbench, the water bottle and cage lid were removed, and the target subject was gently picked up and placed on the bench surface. The investigator held the rat with one hand, lifted the loose skin at the nape of the neck and injected 0.2 cc of sterile saline into the skin pocket, using a 26-gauge needle. The rat was placed back into the cage, which was then returned to the rack. This sequence of events required 20-30 seconds. All

rats showed a significant cardiovascular stress response to the involuntary handling and injection that did not return to baseline within 60 minutes, but rats housed alone had significantly greater increases in heart rate than did rats housed with other rats. This effect could not be confirmed during the tail vein injection procedure, but it was evident during vaginal lavage with females from groups vs. females living alone.

Moncek et al. (2004) compared the stress response of Wistar rats under various conditions to gentle one-minute handling sessions. One set of rats was kept in groups of three or four in barren cages, while the other subjects were taken from groups of 10 animals living in cages five times larger and enriched with various toys, tunnels, swings and a running wheel. The large-group enriched rats showed significantly lower ACTH, corticosterone and adrenaline responses to handling than the small-group nonenriched rats. It is not clear whether the stress-buffering effect was due to the larger number of group members, the more space available to each subject, the enrichment or—as is presumably the case—a combination of these factors.

Belz et al. (2003) studied vein-cannulated, individually housed female Sprague-Dawley rats who lived in 1100-cm<sup>2</sup> cages that were either barren or enriched with rubber toys for gnawing and squares of compressed cotton fiber for shredding. The living space was the same for all animals, but those in enriched cages were not only easier to handle but also showed a significantly lower adrenocorticotropin (ACTH) stress response to intraperitoneal injection. This effect could not be verified in male rats.

Sharp et al. (2005) assessed cardiovascular stress responses to common handling procedures in SH rats, who were individually housed in 930-cm<sup>2</sup> cages that were barren or furnished with a simulated burrow, a feeding enrichment gadget and a shredding-and-nesting item. While blood pressure was not affected by enrichment, heart rate responses to subcutaneous and tail vein injection were significantly lower in enriched vs. non-enriched rats, indicating that enrichment had a stress-buffering effect. This could not be replicated in Sprague-Dawley rats.

Van de Weerd et al. (2002) scored the behavioral responses of RIVM mice kept in groups of eight during routine handling procedures. Animals living in 840-cm<sup>2</sup> cages enriched with a nest box, wood-wool, climbing structures and gnawing blocks showed significantly fewer signs of stress and resistance than animals living in 375-cm<sup>2</sup> unfurnished cages. The stress-buffering effect may

have been due to the enrichment, the larger cage size or a combination of these two factors.

## 2.5. Multi-Tier Caging

### 2.5.1. Variables

Multi-tier caging is the prevailing housing system for rodents and rabbits. It bears intrinsic variables that have the potential to influence research data:

- different level of the living quarters (Figure 4)
- different quality and different quantity of light illuminating the living quarters (Weihe et al., 1969; Ott, 1974; Bellhorn, 1980; Greenman et al., 1982; Clough and Donnelly, 1984; Ader et al., 1991; Kaliste-Korhonen et al., 1995)

Of all the commonly considered environmental factors, light intensity within cages (Clough, 1982) and level of the living environment on the multi-tier cage rack are probably the most variable. The different degrees of illumination resulting from the housing of rodents and rabbits at different levels of the animal room may be one of the explanations for variation in experimental results (Lockard, 1962). If cages are placed at different levels of the room at different distances from the light source, the investigator may be measuring behavioral rather than experimental results (Mulder, 1971, 1976).

In the wild, rodents and rabbits lead a nocturnal or crepuscular life style and change their general activity level as a function of light intensity (Aschoff, 1960; McClearn, 1960; Ross et al., 1966). They show less emotionality, as reflected in decreased defecation and urination in an open field, under low illumination than under high illumination (Ross et al., 1966). There is ample scientific evidence that differences in illumination affect not only behavior and general activity but almost all physiological systems as well, thereby influencing the results of behavioral and physiological experiments and toxicological tests (Chance, 1947; Ross et al., 1966; Porter, 1967; Wurtman, 1967; Weihe et al., 1969; Ott, 1974; Weihe, 1976; Saltarelli and Coppola, 1979).

Rodents and rabbits are terrestrial animals who avoid predators such as humans by retreating under covered structures close to the ground or into burrows under the ground. In laboratories, being confined high above the ground in an upper-row cage without a refuge area is



**Figure 4. Multi-tier caging introduces uncontrolled environmental variables into research data.**

likely to be more disturbing for them than being confined close to the ground in a lower-row cage, which also does not offer a refuge, but is at least relatively dark, hence more secluded.

Many authors do not mention in scientific publications at which level of the room the research subjects were housed (Davis et al., 1973; Lang and Vesell, 1976; Gamble, 1979; Clough, 1982), suggesting that they ignore the possibility that this variable could influence data in their scientific studies. Likewise, there are only a few studies assessing the influence of cage position in a multi-tier rack on behavioral and physiological parameters.

Galef and Sorge (2000) provisioned the cages of individually housed Sprague-Dawley **rats** with PVC tubing. The animals were never seen inside the tubes during the night, but they used them during the day about 60 percent of the time when their cages were located on the top shelf versus only eight percent of the time when their cages were located on the bottom shelf. It stands to reason that the rats used the tubes as protection against overhead light exposure, which was significantly more intense on the top shelf than on the bottom shelf.

Kaliste-Korhonen et al. (1995) observed singly caged Wistar rats from five different shelf levels in an open arena. Rats from the top shelf showed a significantly longer

latency in rearing than rats from lower shelves. This was interpreted as a sign that the animals on the top shelf were better habituated to the high intensity of light to which they were exposed in the open-field test arena.

Izidio et al. (2004) mention that cage position influenced the behavior of singly caged Lewis rats and SH rats in the open-field test, with animals housed in top cages appearing less anxious than those housed in bottom cages.

Ader et al. (1991) assessed emotionality in single- and group-housed NOD **mice**. Mice caged on the top shelf were significantly more emotional—as evidenced by vocalizing, struggling, urinating and defecating—than mice caged on the middle shelf, who in turn were more emotional than mice caged on the bottom shelf. Garner et al. (2004) noticed in mice of various strains, who were kept in different-sized groups in clear plastic cages, that barbering was significantly more severe (higher percentage of body denuded) in upper-row caged groups than in lower-row caged groups. Unfortunately, no ethological data were collected to support the implicit inference that mice caged on upper shelves spend more time barbering than mice caged on lower shelves.

Greenman et al. (1983) found in a large sample of BALB/c mice that animals kept on the top shelf consistently consumed more food, yet had consistently lower body weight gains than animals kept on lower shelves. Greenman et al. (1984) also assessed the distribution of spontaneous and chemically induced tumors in BALB/c mice in relation to the shelf level of their home cages. The animals were assigned to groups of four per cage. Shelf level significantly influenced five of six major spontaneous neoplasms. Lagakos and Mosteller (1981) noticed in CD-1 mice that the incidence of reticulo-endothelial tumors increased conspicuously from the bottom (17 percent) to the top shelf (32 percent), and warned that failure to take shelf level into account in the design of carcinogenicity studies can easily lead to erroneous conclusions.

## 2.5.2. Refinement

It should be emphasized here that US animal welfare regulations stipulate that indoor housing facilities provide lighting that is “uniformly distributed” (United States Department of Agriculture, 2002, p 72 and 81).

Even though it is very costly, cages in a multi-tier arrangement can be illuminated uniformly by mounting light fixtures at the level of each tier on the wall rather than on the ceiling. Behavioral and physiological differences

due to cage position, however, would not be affected by this refinement. As long as the primary enclosures of caged animals are stacked on top of each other in racks, differences in the animals' distances from the ground are unavoidable. This variable is intrinsic to the multi-tier caging system.

## 2.6. Noise

### 2.6.1. Variables

The noise environment of rodents and rabbits is a daily variable that is usually uncontrolled and overlooked in the methodology section of scientific articles, even though it is likely to have important implications not only for the animals' well-being but also for the reliability of research data collected from them (Gamble, 1979, 1982; Pfaff, 1974; Milligan et al., 1993). The pattern of physiological changes elicited by noise in rodents and rabbits is the same as it is in humans. Noise associated with cage cleaning, general maintenance, and especially with construction and remodeling work can be uncomfortable for attending personnel, but it must be overwhelming for the caged animals. The noise can be so intense that certain areas of animal-holding facilities can be officially designated "Hazardous Noise Environments" by Health and Safety Departments. Confined animals may experience the noise not only as annoying to the ears but also as alarming for their sense of security. It is well established in rats, mice, guinea pigs and rabbits that exposure to loud noise is associated with profound alterations in the neural, endocrine and cardiovascular systems (Anthony et al., 1959; Anthony and Harclerode, 1959; Anthony, 1963; Zondek and Tamari, 1967; Lockett, 1970; Armario et al., 1985), which can manifest in mice and rats as potentially fatal seizures (Bevan et al., 1951; Iturrian and Fink, 1968; Wada and Asakura, 1970).

Barrett and Stockham (1996) observed that Wistar rats had elevated plasma corticosterone levels during an experiment on days when the animal attendant had cleaned out the cage racks. A controlled study was then conducted in which singly caged rats were deliberately exposed to ten minutes of loud whistling and talking accompanied by banging of food hoppers, cage doors and fecal dropping trays. This resulted in a significant increase in the rats' corticosterone concentrations.

### 2.6.2. Refinement

There are many strategies to systematically reduce noise in animal facilities that have yet to be implemented. Consultation with a qualified acoustical engineer can lead to specific solutions for all but the most recalcitrant noise abatement problems (Peterson, 1980; Johnson et al., 2005).

Carlton and Richards (2002) took steps to control at least some noise. Using readily available industrial and architectural sound absorption panels, and fitting acoustical covers on electrical motors of cagewashers reduced noticeably the noise level throughout the whole facility by 3-5 decibels (dB), in hallway areas by 5-7 dB, and in cagewash areas by 8-10 dB.

It is also possible to mask noise peaks in animal rooms with relatively loud background noise/music, but the effect on the caged animals is not known (Pfaff and Stecker, 1976).

## 2.7. Summary and Discussion

**Traditional housing, husbandry and handling practices for rodents and rabbits jeopardize not only the welfare of the animals but also the scientific validity of research data collected from them.** Most of these risk factors—confinement, cage cleaning, transfer, restraint and noise—are sources of stress and distress that cannot be avoided in the research laboratory setting, but practices can be refined so that the animals and science are less affected by them. One risk factor—the multi-tier caging system—cannot be refined but could be avoided altogether without adverse effects on the animals and on science.

A good management program not only provides the environment, housing and care that foster the animals' well-being, but also minimizes variables that can influence research data. This will have the added advantage of decreasing the number of animals required to achieve statistical significance in the scientific results (Russell and Burch, 1959; Home Office, 1989; National Research Council, 1996; Öbrink and Reh binder, 1999). Confinement in barren living quarters is probably the most prevalent extraneous variable.

**The distress and fear associated with confinement can be buffered by the presence of one or several companions.** This has been demonstrated in both sexes in rats and mice, and there is good reason to believe that the same holds true for other rodents and female rabbits. Rather than

forcing these animals to permanently live under conditions of social deprivation, thereby jeopardizing their well-being and the validity of research data collected from them, compatible pair- or group-housing should be the norm.

The stress- and distress-mitigating, comforting influence of companionship has been confirmed in several other species including goats (Lyons et al., 1988), sheep (Baldock and Sibly, 1990), chickens (Jones and Merry, 1988), nonhuman primates (Coe et al., 1982; Shively et al., 1989; Coelho et al., 1991), and human primates (Bovard, 1959; Epley, 1974, Kawachi and Berkman, 2001). It is reasonable to assume that being able to engage in positive social behaviors contributes to the well-being of any social animal (Institute for Laboratory Animal Research, 1992), including rodents and rabbits.

**Distress and fear due to confinement can also be ameliorated by increasing the complexity of the living space.** Objects and structures that can be used as hiding places are particularly effective in enhancing the confined subjects' ability to cope with distress by taking refuge in a secluded place. This has been shown in rats and mice, both in single- and in group-housed animals. It remains to be investigated whether confinement distress can also be buffered by specific environmental modifications in the case of hamsters, gerbils, guinea pigs and rabbits.

**Maladaptive behaviors are generally regarded as objective signs of inadequate housing conditions.** Rodents and rabbits develop such behaviors even in groups kept in large, well-furnished enclosures. This underscores the fact that enforced permanent confinement is an intrinsic stressor for them.

Behavioral problems become particularly evident during the animals' activity phase, which in rodents and rabbits is the night. They may be overlooked completely during the day when the animals sleep. To assess the behavioral health of nocturnal animals, it is essential to conduct observations during the night without being a source of distraction or disturbance. Such observations are lacking in the literature, so it is very possible that behavioral pathologies are much more common in rodents and rabbits than has been usually assumed.

**In rodents, the incidence and the frequency of maladaptive behaviors can be reduced—but not eliminated—by the provision of cover-providing structures and substrates.** These environmental modifications are likely to increase the animals' sense of security and well-being, thereby decreasing their need to

engage in bizarre behavior patterns that may help them cope with inner tension arising from inadequate living conditions.

In rabbits, maladaptive behaviors are best treated with companionship and/or provision of hay or straw. These additions to the environment offer species-appropriate distraction, thereby decreasing the time that the animals could spend engaging in maladaptive behaviors.

Preliminary evidence suggests that some maladaptive behaviors can be “imprinted” in the subject's neurophysiological system (Garner, 2005). It has been demonstrated in voles (Ödberg, 1987), pigs (Arellano, 1992), horses (McAfee et al., 2002), chickens (Jones et al., 2004), nonhuman primates (Chamove et al., 1984; Kessel and Brent, 1998), human primates (Christenson and Mansueto, 1999) and other species (Moon-Fanelli et al., 1999) that once established, these pathologies are extremely resistant to treatment. Species-appropriate environmental modifications may alleviate but not eradicate them, while pharmacological intervention may stop them only temporarily.

It should be possible to create and test housing arrangements and husbandry practices for rodents and rabbits that are so species-appropriate that the animals have no reason to develop maladaptive behaviors while they are young, and to keep them under conditions that do not prompt maladaptive behaviors once they are mature. Only then will it be possible to investigate specific factors to which the animals have difficulties adapting or to which they cannot adjust at all.

**Aggression among social partners and defensive aggression against handling personnel is a common problem that has received surprisingly little attention in the literature.** The provision of hiding options from threatening partners decreases the risk of injurious fight wounds not only in mice and hamsters, but probably also in gerbils, guinea pigs, and perhaps also in rabbits. The aggression-mitigating effect of structures that allow individuals to break visual contact with opponents has also been documented in pigs and nonhuman primates (Erwin, 1977; Waran and Broom, 1993; Maninger et al., 1998; Westergaard et al., 1999).

That group-housed hamsters are less aggressive than singly caged hamsters when being handled by personnel has important practical implications that also deserve exploration in other rodents and rabbits.

**The scientific documentation that social compan-**

**ionship and species-appropriate furniture enhance normal brain function and stimulate brain tissue integrity in rodents highlights the folly of not housing the animals in compatible social arrangements in complex primary enclosures.** A social animal who is permanently kept alone in a barren environment is literally crippled both neurophysiologically and ethologically. It is questionable whether species-representative data can even be obtained from such a subject.

**The cleaning of their cages is a variable that regularly affects rodents and rabbits to a varying extent.** Groups of male mice tend to react to this disturbance not only with stress but with conspicuous outbursts of serious aggression. This is probably the reason that refinement attempts have been focused on mice, while rats, hamsters, gerbils, guinea pigs and rabbits have been largely ignored.

Transferring male mice into completely cleaned cages with mouse-odor-free structures for escape and some old *nesting* material is probably the best option for minimizing inter-male aggression and mitigating the stress associated with this husbandry procedure. It is important to find out if similar techniques can be applied to other rodents and rabbits.

**The stress resulting from transfer to an unfamiliar location and separation from cagemates is significant, but too little research has been conducted to find out whether this stress can be mitigated or even avoided.** Transferring an animal to an unfamiliar location or separating an animal from cagemates is often not necessary. Many non-invasive procedures can be carried out in the subject's home cage, and if the subject lives in a group setting he/she can be separated in an extra cage that still allows continual communication with the familiar group. There is also the possibility of moving an animal along with one or several companions, who will serve as a stress buffer in the unfamiliar location where the experiment takes place. These options need to be explored more systematically. They are likely to refine experimental methodology by reducing or eliminating the anxiety and fear of the subject.

**The few studies that address the possibility of habituation suggest that the animals may adapt to being picked up and gently handled, but not to being forcibly restrained.** There are three options available to buffer the stress associated with restraint:

a. Positive reinforcement training has been applied with amazing success for oral drug administration in rabbits and rats, but no stress-sensitive parameters were measured to see whether cooperative animals are actually less stressed than resisting animals. Studies with nonhuman primates have demonstrated that subjects show no or a significantly lower corticosterone response to blood collection when they have been trained to cooperate during this procedure, compared to subjects who resist the enforced restraint during venipuncture (Elvidge et al., 1976; Reinhardt, 1996; Bentson et al., 2003; Videan et al., 2005). The training protocol for oral gavage developed for rabbits and rats can probably be adapted to other species.

Positive reinforcement training techniques for injection, blood collection and weighing need to be explored. They could benefit both the animals and scientific methodology.

b. A few studies with rats have demonstrated that the physiological stress reactions to common procedures can be buffered—though not eliminated—by housing the animals in a social setting rather than alone.

The stress-buffering effect of a social partner during procedures is not receiving sufficient attention in the literature. Companionship may offer a very simple, inexpensive, and practical means of minimizing the otherwise uncontrolled variable of restraint stress.

c. Species-appropriate environmental enrichment can also buffer restraint stress in individually caged rats. This encouraging finding deserves special attention and also exploration in other rodent species and rabbits.

**An animal caged on the top shelf lives in an environment that is much higher and receives different illumination than one caged on the bottom shelf.** It is difficult to understand why this variable is generally overlooked. The few observations published strongly indicate that shelf level does have an impact, probably not only in rats and mice, but also in multi-tier caged animals in general. Ignoring this variable, because more animals can be kept in a room when the cages are stacked on top of

each other than when they are arranged at the same level of the room, contravenes scientifically sound research methodology.

It is a legal requirement and professional recommendation that laboratories should provide uniformly distributed illumination of sufficient intensity to permit routine inspection and adequate housekeeping practices, including the bottom-most cages in a rack (National Research Council, 1996; United States Department of Agriculture, 2002). No advice is given as to how this important stipulation can actually be met. Rotating cage position relative to the light source (National Research Council, 1996) will rotate the two variables—distance from light source and distance from floor—between the subjects, but it does not address the real problem of minimizing or eliminating them.

**Noise is another variable that is commonly overlooked, even though there is scientific evidence that noise associated with husbandry procedures can be a source of stress, and that exposure to loud noise can affect the neuroendocrine system.** There is the possibility of controlling some noise with sound absorption panels, but this option has not yet been systematically implemented, nor has its effect on the animals' physiological systems been studied.

Rodents and rabbits can hear sound frequencies that are inaudible to humans but which may affect the animals nonetheless. This circumstance has to be taken into consideration in efforts to control the effect of noise, within and outside the human range of sound perception, on research data.



# 3. ENVIRONMENTAL IMPROVEMENTS

Environmental Improvements are changes in traditional housing conditions that can promote the physical and behavioral well-being of the confined subject.

Well-being is the subjective state of contentment. The well-being of a subject is enhanced when basic needs for physical health, behavioral health and comfort are met.

## 3.1. Environmental Necessities

Environmental Necessities are husbandry factors that address basic species-specific needs of the confined subject.

### 3.1.1. Flooring and Bedding Material

Grover-Johnson and Spencer (1981) found, in Sprague-Dawley **rats** who were kept all their lives on wire mesh flooring, that the distal sciatic/tibial/plantar nerve complex developed striking morphological abnormalities, presumably as a result of the rat-inadequate flooring condition of the cages. Peace and Singer (2001) assessed the clinical records of Sprague-Dawley rats who had been kept for more than one year either in wire-bottom cages or in large solid-bottom cages with woodchip bedding. The incidence of clinically observed foot lesions (callus, ulcer/crust, swelling) was 2 percent in both females and males kept on a solid floor, compared with 30 percent in females and 92 percent in males kept on a wire mesh.

Bradshaw and Poling (1991) tested Sprague-Dawley rats in divided wire-bottom cages where one half was empty, and the other half was covered with plywood or a layer of woodchips that were prevented from falling through the wire mesh by a pan attached to the cage bottoms. The rats avoided the wire mesh and showed a significant preference for the side of the cage with plywood (72 percent) or with woodchips (71 percent). Manser et al. (1995) showed that Sprague-Dawley rats raised on wire mesh flooring strongly prefer a solid floor when they are given the choice, and they are prepared to make considerable efforts to reach a solid floor when they want to rest (Manser et al., 1996). They will spend most of the time (81 percent) in such a cage and will stay away from the mesh floor cage to which they were originally habituated (Manser et al., 1996). Blom et al.

(1993) confirmed in Wistar rats, that individuals avoid wire mesh flooring.

Krohn et al. (2003) measured telemetrically two cardiovascular stress parameters in pair-housed Sprague-Dawley rats. Blood pressure and heart rate were significantly higher during both day and night when the rats were kept on wire mesh or solid plastic floors compared with a layer of woodchips. This suggests that hard floors are relatively stressful for rats.

Mulder (1974a), Blom et al. (1993) and Ras et al. (2002) found that Sprague-Dawley and Wistar rats have a strong preference for large, fibrous bedding material such as woodchips over corncob litter and sawdust.

Blom et al. (1996) tested the preference of C57BL/6 and BALB/c **mice** for a cage with wire mesh flooring vs. solid flooring with various beddings. The animals avoided the wire mesh but showed a significant preference for solid flooring with bedding. Shredded filter paper was the most attractive substrate, followed by woodchips. Bedding material of small particles was relatively unattractive, probably because it was unsuitable for nest building and potentially irritating to the eyes (Figure 5). Eskola and Kaliste-Korhonen (1999) also noticed that BALB/c and C57BL/6 mice clearly prefer aspen wood-wool to “conventional bedding” (presumably aspen chips; Eskola et al., 1999), because they can use it both as bedding and convert it into comfortable nests. Paper tissues and paper-based structures that can be shredded provide ideal bedding and nesting material for mice (Figure 6).

Port and Kaltenbach (1969) found in SCH:ARSHA mice a significantly increased preweaning mortality when the animals were kept on corncob when compared with pine sawdust bedding (22 vs. 13 percent).

Mulder (1974b, 1975) noticed that female SCH:ARSHA mice invariably prefer woodchips over small particle bedding materials derived from corncob, dehydrated alfalfa or clay.

Smith et al. (2000) furnished the wire-bottom cages of individually housed CD-1 mice each with a 5 x 5-cm rayon/polyester gauze. This pad was changed weekly throughout a test period of 52 weeks. The study confirmed that the mice preferred to rest on the gauze pad rather than



**Figure 5. The traditional small-particle bedding substrate does not allow mice to construct nests that could insulate them from aversive environmental factors.**

on the wire mesh. Whenever they were undisturbed, the mice were in contact with their pads. Usually they pulled at the threads without breaking them, and fluffed them into perfect material for nest construction (Watson, 1993).

Pettijohn and Barkes (1978) observed **gerbils** who were given the choice of entering equally-sized compartments offering plastic flooring, woodchips, sand, a sheet of newspaper or a terrycloth towel. Both sexes had a significant preference for sand and a secondary preference for woodchips. The sand was particularly attractive, eliciting digging and gerbil-typical sand bathing, which serves to remove excess body oils from the fur.

Arnold and Estep (1994) tested golden **hamsters**



**Figure 6. Mice need large, fibrous material for the construction of their nests.**

in an apparatus that gave them simultaneous access to a wire-floored 510-cm<sup>2</sup> standard cage and a solid-floored 450-cm<sup>2</sup> cage with corncob litter. Subjects spent significantly more time (70 percent) on the solid floor with litter, even though this implied a reduction in floor space.

Fullerton and Gilliat (1965) examined **guinea pigs** housed singly in 1000-cm<sup>2</sup> cages and noticed pressure neuropathies in the plantar nerves in 94 percent of 16 animals kept on wire mesh floors, in contrast to 19 percent of 16 animals kept on solid floors covered with a deep layer of sawdust.

Turner et al. (1992) observed Dutch x French Lop **rabbits** in a test cage offering a choice of two different substrates. The rabbits avoided sawdust, woodchips and bare concrete floor but showed a significant and equal preference for both straw and shredded paper. Straw was specially favored, and the animals spent more time nibbling and manipulating it than they did paper (Figure 7).

### 3.1.2. Shelter, Burrows, Nest Boxes and Nesting Material

Bradshaw and Poling (1991) tested the preference of Sprague-Dawley **rats** for equally-sized living quarters that were either barren or furnished with a PCV tube 7.6 cm in diameter, or with paper towels. The rats showed little interest in the plastic tube but a strong preference for paper towels, which they immediately used to construct comfortable nests.

Patterson-Kane et al. (2001) gave Hooded Norway rats the choice of spending time in a barren cage versus identical cages provisioned either with four sheets of tissue paper (*sic*), one handful of shredded paper, a PVC tube (8-cm diameter), a plastic cylinder (15-cm diameter) or a coffee tin with an 8-cm-diameter entrance hole. Female and male rats showed significant preferences over the barren cage only for shredded paper and the tin box. The other enrichment options elicited negligible attention.

Nolen and Alexander (1966) compared two different nesting materials and noticed that Sprague-Dawley rats weaned significantly more pups per litter (10.8) when they were kept in cages furnished with shredded paper vs. woodchips (7.0). Access to shredded paper decreased infant mortality from 45 to three percent. Females with woodchips simply “dried up”. Those with shredded paper built nests and had full milk glands, as reflected in substantially higher lactation indices. The paper nests allowed them to burrow

Maureen Harpden, Hoffmann-La Roche, Inc., Nutley, NJ, USA. ©Roche Laboratories Inc., 2005

and insulate themselves from disturbing environmental factors and also to create an optimal microclimate for their pups (Figure 8). Norris and Adams (1976) found in CFHB rats, that wood-wool may be an even more appropriate nesting material. Females with access to wood-wool reared a much higher proportion of their young (82 percent of 1182) than females with paper tissues (40 percent of 791). Rats with wood-wool constructed more elaborate, and probably more species-typical, nests than rats with paper tissues. Animals who had access to wood-wool for a long period of time (500 days), however, were susceptible to subcutaneous tumors and nasal scabbing.

Galef and Sorge (2000) examined the usefulness of 7.5-cm-diameter PCV tubes as shelters for singly caged Long-Evan rats. While males were seen inside the tubes only rarely, females used the tubes approximately 36 percent of the time during the night and 20 percent of the time during the day.

Townsend (1997) demonstrated that male Wistar rats have a strong preference for living quarters that contain an upturned mouse cage (33 x 15 x 13 cm). The animals used such a structure as shelter, an object to sit on, gnaw and move around. The mouse cage also increased the wall surface, thereby fostering the animals' urge to move close to walls (thigmotaxis, "wall-hugging") rather than on unprotected terrain.

Patterson-Kane (2003) tested female Wistar rats in a T-maze for preference of a barren cage versus a cage of the same size, furnished with one of seven different shelters the size of a mouse cage. The rats had a consistent (73-95 percent) preference for the cage containing the shelter. They preferred shelters made from plastic or tin to cardboard, opaque shelters to translucent shelters and enclosed shelters with one 8-cm<sup>2</sup> entrance hole to those open on one side.

When their cages are furnished with appropriate shelters, rats will spend about 85 percent of the time in them during the day—when the animals rest—and 25 percent of the time during the night—when the animals are active (Collier et al., 1990; Townsend, 1997; Eskola et al., 1999; Saad et al., 2004).

Manser et al. (1998a) tested the suitability of 20 x 12.5 x 12-cm plastic boxes of different transparencies and design, and various nest materials, in male Sprague-Dawley rats. All three boxes were used for resting throughout most the day and part of the night. The rats favored an opaque nest box over a transparent one and a nest box with enclosed



Gun-Dore, 1987

**Figure 7. Straw is a particularly attractive bedding and foraging material for rabbits. Cardboard boxes provide look-out posts and shelters.**

corners (one 9 x 10-cm entrance) over one without front and back walls. They strongly preferred soft paper strips (0.5-1.0 cm wide and 40 cm long) over coarse paper strips, woodchips and nestlets. They did not build nests with the paper strips but spent an average of 93 percent of the time manipulating and gnawing them during the day and 77 percent of the time during the night.

Jegstrup et al. (2005) demonstrated, in three inbred strains (BN, BDIX, LEW) of pair-housed male rats, that naïve animals who have never had access to any kind of nesting material *do* build nests if the correct stimuli are provided (Figure 9a & b). The animals were housed in 48 x 38 x 20-cm cages with aspen chips bedding, each furnished



Lawlor, 1987

**Figure 8. Appropriate nesting material—here shredded paper—is a necessity for rats to build relatively comfortable nests for themselves and for their offspring.**

with a roofed wooden nest box measuring 28.5 x 20.5 x 12.5 cm. The box had entrance holes on two sides, each of 6-cm diameter. Wood-wool and autoclaved straw—the material of choice for nest-building in wild rats (Calhoun, 1962)—were placed outside the box. Every two weeks the nest was removed and the box, along with the two rats, transferred to a new cage. Additional fresh bedding material and straw were added twice a week, and wood-wool once a week. The nest box was machine-washed every two months. All animals built a new nest in the box during the two-week period between cage changing procedures. They would begin with the removal of all aspen chips from the nest box, continue with the collection of straw and wood-wool and finally form a mat inside the empty nest box. This



**Figure 9a & b. When they are provisioned with a rat-adequate shelter and rat-adequate nesting material (Figure 9a) male rats will move all the substrate into the shelter and build a well-formed nest (Figure 9b) even when they have never before been exposed to a shelter nor nesting material.**



mat could be several centimeters thick and was then arranged into a cup-shape nest. The nest consisted of both straw and wood-wool fibers, with straw making up the major part of the structure. Constructing their own nests not only allowed the rats to engage in a species-typical behavior, which presumably enhanced their feeling of security, but also reduced the incidence of aggressive interactions (Jegstrup and Ritskes-Hoitinga, 2004).

Van de Weerd et al. (1998a, 1998b) assessed in choice tests the preference of C57BL/6 and BALB/c mice for barren versus same-sized cages furnished with (a) a rectangular, 8 x 10 x 6-cm nest box of various qualities or (b) different types of nesting material. Pilot studies by Buhot-Averseng (1981) have shown that outbred mice have a preference for rectangular, narrow, roofless frames with perforated walls. In Van de Weerd's studies, both strains and both sexes showed significant preferences for a cage containing a nest box or nest material over an empty cage. Heinzmann et al. (1998) made a similar observation in group-housed female Him:OF1 mice. Van de Weerd's mice strongly preferred nest boxes made of materials that allowed olfactory and visual cues to pass, e.g., grid metal or perforated metal. They stayed away from dark nest boxes made of sheet metal or gray PVC. Paper tissue was preferred over paper towel as nesting material. When given the choice the mice spent an average of more than 69 percent of the 24-hour day in the cage with the paper tissue, compared with less than 25 percent in the cage with the most preferred nest box.

These observations demonstrate the importance for mice to construct their *own* nests according to their comfort and microclimatic needs. Prefabricated structures have their place as a refuge in the event of an alarming situation, but not as a mouse-adequate sleeping site.

Key and Hewett (2002) observed same-sex trios of BALB/c mice who each had access to a dark triangular plastic shelter with one entrance at the front and a second entrance/exit in the roof. Mice with the shelter showed no difference in general activity, or in agonistic behavior, but they engaged in bar-gnawing significantly *more* than control mice without shelter. These findings were confirmed in both sexes and they suggest that this particular shelter did not meet mouse-specific comfort requirements.

When appropriate nesting material is put on the cage lid, mice will not hesitate to pull it into the cage and start building a nest (Lynch and Hegmann, 1972). Van de Weerd et al. (1997) inferred from choice tests that

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C57BL/6 and BALB/c mice of both sexes prefer soft paper over woodchips or wood-wool. They will spend about 15 percent of the 24-hour day dragging the material into the right position and building a nest, in which they will spend about 50 percent of the time sleeping and grooming.

Porter and Lane-Petter (1965) reported that mice had a lower preweaning mortality when they had access to shredded paper to build nests than when they were kept on woodchips. Obviously, a nest provides a quieter rearing environment and a much better regulated microclimate than woodchip bedding.

Hobbs et al. (1997) videotaped male mice kept in groups of four in 260-cm<sup>2</sup> cages furnished with nestlets. This cellulose nesting material was contacted by CD-1 mice 89 percent of the time, by DBA/s mice 65 percent and by B6C3F1 mice 72 percent of the time.

Nestlets are attractive for mice, but they can be a health hazard. Bazille et al. (2001) noticed an increased prevalence of conjunctivitis in Hsd:Athymic Nude-nu mice who were provided with nestlets. The conjunctivitis resolved completely when the nestlets were replaced with paper towels as nesting material.

Van Loo et al. (2005) tested the preference of groups of three or four female BALB/c, C3H/HeNHsd and C57BL/6 mice for an approximately 15 x 9 x 6-cm paper-based, triangular nest box with a 4-cm-wide access hole, or a similarly shaped and sized red transparent plastic nest box with a 5 x 3-cm opening on the long side and another triangular opening in the top of the box (Figures 10 & 11b). All three strains showed a significant preference for the paper box, which had the advantage of being much lighter (20 g) than the plastic box (95 g), allowing the mice to move the whole structure around and change the position of its entrance. The plastic box was too heavy for such maneuvering and hence never changed its place. The mice also gnawed the paper box, occasionally nibbled an extra hole in the side or shredded part of its walls, using the shreds to strengthen the nest. They could not do this with the plastic box. All groups of mice slept inside the paper box, but never in the plastic box. If they chose to sleep in the cage that contained the plastic box, they did so in the sawdust *outside* the box. When paper tissues were provided, the mice dragged the material into the paper box and built a nest, but they never attempted to build a nest in the plastic box (Figures 6 & 10).

Mice may investigate and climb on plastic shelters but will be reluctant to use them as nests. They will rather



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**Figure 10. Paper-based shelters and soft paper are species-appropriate materials for mice to build nests in which they can retreat and sleep.**

sleep outside than in a plastic shelter (Figure 11a). When given the choice, they will build their own nest and sleep in it rather than make use of a prefabricated, indestructible “mouse house” (Figure 11b).

Sherwin (1996) concluded, from a series of preference tests with individually caged male TO mice, that the animals were less motivated to use tubes as sleeping sites than to use sawdust. No preference for tubes was found, regardless of their shape, length, openness, opacity and width. A tube can be useful as a refuge when the animals are startled, but it is not a good substitute for a nest.

Sherwin et al. (2004) found that BALB/c mice are more motivated to burrow rather than run through tunnels. They will spend about ten percent of the 24-hour day



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**Figures 11a. Mice investigating a plastic shelter but resting outside rather than in the shelter.**



Pascalie van Loon, Utrecht University

**Figures 11b. Mice have built their own nest rather than made use of a prefabricated, indestructible “mouse house.”**

burrowing if they are provided with a suitable substrate such as compacted dampened peat. Throughout a study period of nine days, the mice did not decrease their burrowing activity, suggesting that the expression of this behavior—like nestbuilding—also fulfills a strong biological need.

Waiblinger and König (2004) furnished the cages of **gerbil** families with an artificial burrow system consisting of a nest box and an angled access tube. In a choice test, the animals clearly preferred a dark burrow system over a transparent one. They spent about 80 percent of the 24-hour day in the dark burrow. The importance of a dark burrow is in agreement with the fact that gerbils prefer partially darkened cages over transparent standard cages (Van den Broek et al., 1995).



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**Figure 12. Hamsters have a keen interest in soft paper and will construct their nests with it, preferentially in a shelter.**

Jansen (1969) showed in golden **hamsters** that both females and males quickly learn to press a bar to obtain small strips of paper (Figure 12). They would typically push the strips into their cheek pouches, return to their home cages, retrieve the paper and do some nestbuilding, then return to the test chamber, press again and continue with the hoarding of this highly valued material. These observations demonstrate that paper serves as a potent positive reinforcement for hamsters, comparable to food and water.

Otoni and Ades (1991) showed that hamsters prefer a dark shelter over a transparent one (Figure 13), and a relatively large shelter over a small one (730 cm<sup>2</sup> x 10 cm vs. 320 cm<sup>2</sup> x 10 cm).

There are no published data on species-appropriate shelters for **guinea pigs**, even though there is a general consensus that the animals are easily startled and, therefore, need adequate provision of cover during alarming situations, which are unavoidable in the research laboratory (Gray, 1988; Reinhardt, 2002; Banjanin et al., 2004; Ottesen et al., 2004; Figure 14).

Hansen and Berthelsen (2000) furnished the 3540-cm<sup>2</sup> single-cages of NZW and French Lop **rabbits** with 44 x 25 x 19 cm wooden boxes with perforated plastic floors and roofs (Figure 15). After a 3-month habituation period, the rabbits hardly made use of the box (about 1 percent of the time) as a place for rest or escape but spent about 56 percent of the video-recorded time on its roof. Obviously, the animals had no need for a shelter but benefited from an elevated look-out.

### 3.1.3. Social Housing

Patterson-Kane et al. (2002) showed that individually caged female Hooded Norway **rats** will readily work, by pressing a lever, to get access to a cage containing three other female rats, but not to get access to a cage that is empty but larger than their home cage. Companionship, evidently, was of greater importance for them than unstructured space. When the rats could choose between cages containing one, three, five or 11 rats and the stocking density was kept constant (20 cm<sup>2</sup> per subject), they showed a clear preference for five rats (Patterson-Kane et al., 2004).

Moncek et al. (2004) reported significantly higher plasma concentrations of corticosterone in male Wistar rats kept in groups of ten in large cages (500 cm<sup>2</sup>/subject) furnished with toys, tunnels, swings and running wheels than in rats kept in groups of three or four in relatively



**Figure 13. A dark nestbox with nesting material should not be regarded as environmental enrichment but as basic furniture, ensuring basic comfort needs of the caged hamster.**

small unfurnished cages (290 cm<sup>2</sup>/subject). Unfortunately, it is not made clear whether the difference in corticosterone levels was related to the difference in group size, cage size or cage equipment. Living with more partners in a larger and enriched cage probably increased the overall level of the subjects' activity, which may have resulted in a biologically normal, intermittent endocrine activation reflecting eustress.

Brown and Gunberg (1995) compared the stress status of female and male Wistar rats housed either alone in a large (1010 cm<sup>2</sup>) barren cage or in small groups of four under crowded conditions in a much smaller (100 cm<sup>2</sup>/female; 160 cm<sup>2</sup>/male) barren cage. Female rats had significantly higher plasma corticosterone levels when they were caged singly than when they shared a small cage with three other females, but the opposite was true



Staff of the University of Birmingham

**Figure 14. Guinea pigs avoid unprotected, open areas. Shelters, PVC tubes and hay allow them to “duck under” during alarming situations.**



Natasha Down, York University, Toronto, Canada

**Figure 15. There are no data published assessing the effect of shelters on the compatibility of group-housed rabbits.**

for males. Male rats had relatively low corticosterone levels when they were alone but higher levels when they had to share a small cage with three other males. While the key stressor for female rats was social deprivation, crowding was the key stressor for males.

Rank-related conflicts are not common in caged rats (Grant and Chance, 1958), and there are no published reports of serious problems associated with overt aggression in same-sex pairs or groups. However, low-ranking group members may experience social distress under the given constraint of artificial confinement and competition for

resources (Blanchard et al., 1995; Hurst et al., 1996; Hurst et al., 1999).

Van Loo et al. (2001) gave male BALB/c **mice** the choice of staying in either an empty cage or in a divided cage of the same size with one half already occupied by an unfamiliar male mouse who could be seen and smelled through a transparent wall with holes. The separating wall served as a precaution against overt aggression for which male mice are notorious. The test mice showed an overall preference for the inhabited cage, which indicates that “even” male mice prefer the company of another male mouse over being alone.

The social housing of male mice can jeopardize the safety of the animals. There is usually one dominant male who vigorously defends his territorial boundaries (Mackintosh, 1970). In order to avoid conflicts, subordinate males try to keep at a safe distance or simply leave the territory. The constraints of confinement usually make this impossible, and considerable, often sustained fighting between males becomes a common event. Subordinate males never retaliate when attacked by the dominant mouse but may demonstrate distress behavior such as flight attempts and submissive/defensive postures, often accompanied by squealing. Bolam (2005) reported a 25 percent incidence of injuries in 70 male CD-1 mice who were housed in pairs or trios. Due to aggressive incompatibility 8 percent of the animals had to be separated during a 10-month study. Although fighting does not necessarily lead to serious injury, the dominant male may fiercely attack over and over again and even kill other males. Emond et al. (2003) report a case in which 0.3 percent of the annual population of 37,566 group-housed male CD-1 mice had to be removed due to serious injuries. Out of concern, animal care technicians started separating dominant males who threatened, attacked or chased other males. As a result, only 0.13 percent of the next annual population of 25,802 male mice had to be removed due to serious wounds resulting from aggression.

Haemisch and Gärtner (1994) and Bergman et al. (1994/95) demonstrated in trios of male DBA/2J and HLG/Zte mice that the provision of vertical dividers may increase rather than decrease aggressive conflicts, presumably because the dividers can serve as territorial boundaries for the most dominant male, while providing subordinate animals insufficient escape options. Marashi et al. (2003) kept male CS mice in groups of four either in barren 835-cm<sup>2</sup> standard cages or in spacious 4000-cm<sup>2</sup> terraria

that were enriched with several climbing structures, hemp ropes, several platforms at different levels and a ladder. Mice in the enriched environment were engaged in agonistic conflicts significantly *more* often than non-enriched mice, presumably because the various structures in the enriched terraria promoted the establishment of territories designated by visual landmark boundaries. The increased level of territorial disputes was reflected in increased stress, as measured by significantly higher plasma levels of glucocorticoids compared with mice living in barren cages. However, this was counterbalanced by significantly higher rates of positive social behaviors—sitting together with body contact, playing, and allogrooming—in the enriched mice.

Aggression-related problems with the social housing of female mice have not been reported in the literature.

Arnold and Estep (1990) observed male golden **hamsters** placed with another littermate in a specially designed enclosure that allowed them to stay away from, or in proximity to each other. During 46-hour test sessions individuals spent 67 percent of the time in proximity to each other. Arnold and Gillaspay (1994) confirmed these findings in a follow-up study of female hamsters, and concluded that hamsters do not prefer social isolation regardless of the fact that social housing is typically associated with overt aggression, and hence may be more stressful for the animals.

The social needs of **guinea pigs**, especially in relation to gender, have not yet been assessed. There is, however, a general consensus that guinea pigs should always be housed in a social environment (Lawlor, 1997; North, 1999; Raje and Stewart, 2000; Banjanin et al., 2004; Ottesen et al., 2004; Sachser, 2004).

Aggressive interactions among females are rare and of no serious consequences under normal conditions (Reinhardt, 1971). Overt aggression can be a problem in males. Agass and Ruffle (2005) inserted a clear plastic partition in the cages of groups of four. Each group was divided into two pairs who maintained uninterrupted visual contact with each other. The authors do not provide data but make the statement that implementing these cage dividers “considerably reduced the incidence of bullying... and there have been no further significant incidents of biting or aggression within pairs.” Beer and Sacher (1992) compared the plasma glucocorticoid levels of males kept in pairs and in groups of six or twelve. Average corticosterone concentrations were significantly lower in pairs, suggesting

that group-housed males were more stressed.

Whary et al. (1993) kept female NZW rabbits individually in 3600-cm<sup>2</sup> barren cages or as a group of eight in a floor pen (3750 cm<sup>2</sup>/subject) furnished with a resting shelf, a litter box and a PVC tube serving as potential shelter. Both categories of rabbits were monitored on a regular schedule over a 12-week period. Single and group-housed animals did not show significant differences in immune responses, plasma corticosterone concentration and growth rate, indicating that research-sensitive physiological parameters measured in the conventional single-housing system were not affected by the unconventional group-housing arrangement. Group-housed rabbits did not show either an increased incidence of infectious disease or any injuries traceable to aggression. Group-housed rabbits benefited from the increased available space and were frequently observed lying in the rabbit-typical full stretched-out, lateral recumbent position, which singly caged rabbits usually cannot adopt due to insufficient space (Gunn-Dore, 1997).

Turner et al. (1997) kept does in groups of four or five in large pens (16,700 cm<sup>2</sup>/subject) provisioned with straw bedding, cardboard boxes and raised shelves. High- and low-ranking members of each group were identified and their immune status compared. No evidence of immunosuppression related to social rank was found either in NZW or Dutch x California breeds.

Huls et al. (1991) observed NZW does who were pair-housed in two interconnected 2580 cm<sup>2</sup> cages. Partners spent on average 88 percent of the observations in the same half of the cage and were in direct body contact with each other about 20 percent of the time. Brooks et al. (1993) conducted a similar study in which adult pair-housed NZW does also chose to stay together in the same half of a double cage 90 percent of the time. Held et al. (1995) gave individual members of groups of four adult female NZW or Dutch x California rabbits the choice of (a) joining the other three group members in a large pen or (b) moving into a solitary, yet equally large and equally furnished pen. Both low-ranking and high-ranking does showed a consistent, albeit moderate, preference not for the pen with the other companions, but for the solitary pen. Together, these findings indicate that female rabbits do have a strong affinity to each other, but that they want to be alone at times.

Aggression is a normal social behavior that has its intrinsic value in establishing and confirming dominance-subordination relationships. However, within the constraints

of artificial confinement social aggression can become a problem, because the animals are forced to stay in close proximity all the time.

Domesticated rabbits are remarkably docile with humans and demonstrate hardly any fear responses, but both sexes tend to be more aggressive among themselves than their wild counterparts are (Kraft, 1979a; Kraft, 1979b). Rabbits should be housed in a social setting, but it can be a challenge to minimize the risk of aggressive incompatibility (Morton et al., 1993).

Tamburrino et al. (1999) assessed the clinical records of 135 sexually mature female rabbits of unspecified breed. The animals were housed in groups of five in pens (5200 cm<sup>2</sup>/rabbit) that were covered by a 10-cm layer of woodchips topped by 14-cm of straw. Each pen contained half of a 58-cm-diameter plastic barrel shelter. Over the course of a nine-month period 23 (17 percent) rabbits sustained minor, and two (1.5 percent) sustained major fight wounds.

Based on the clinical records of several thousand group-housed female NZW rabbits, Love and Hammond (1991) and Love (1994) offer the following recommendations to minimize injurious aggression:

- a. Group sizes of four to eight rabbits work well if the groups are to remain together for long periods.
- b. It is highly desirable to establish a new group with young animals who have not yet reached the age of puberty.
- c. The animals in a group should be of the same age, but not necessarily from the same litter.

An anecdotal report by Howard et al. (1999) suggests that the provision of a visual barrier behind which subjects can “escape” and tubes in which subjects can hide minimizes aggression among group-housed, probably female Dwarf Lop rabbits. Held et al. (1994) concluded from observations of a group of 20 NZW and a group of 20 Dutch x California does kept in 3-m<sup>2</sup> floor pens that raised platforms reduced agonistic interactions by allowing the pursued animals to move out of sight. Statistical data analysis, however, did not support this presumed effect.

Chu et al. (2004) recorded the behavior of non-littermate female NZW rabbit pairs over a five-month period as the animals matured from juveniles to mature does. The animals were kept in 9270-cm<sup>2</sup> cages *without* structures

that would have allowed them to get away from each other. Companions spent on average about 27 percent of the time in physical contact with each other. One of the four study pairs, however, had to be separated due to persistent aggression resulting in serious bite wounds at the end of the study, after the two partners had become sexually mature. Bigler and Oester (1994) witnessed injurious aggression in one of eight pairs during a seven-month study period. Nevalainen et al. (2003) kept littermate female NZW rabbit pairs in 7200-cm<sup>2</sup> cages that were furnished with a raised shelf but *no* structures for privacy. During a 4.5-month study occasional fighting between cage companions was seen, but after the animals had reached sexual maturity more frequent and more serious fighting necessitated early termination of the study.

Once they reach sexual maturity male rabbits become even more intolerant of each other than females. This makes their housing in a social setting not advisable (Morton et al., 1993; Love, 1994).

Kalagassy et al. (1999) castrated Flemish Giant x Giant Chinchilla prepubertal males to see whether this would make them more suitable for social housing. A total of 10 intact and 12 castrated male littermates were housed in pairs in 12,700-cm<sup>2</sup> cages each furnished with a wooden nest box and provisioned regularly with vegetables and hay. Behavioral observations revealed that castrated males engaged in aggression significantly less often (0.02 vs. 0.3 percent), but spent significantly more time (41 vs. 19 percent) resting in body contact with each other than intact males. While three of the five intact male pairs had to be separated due to injurious aggression, none of the six castrated male pairs required separation during an unspecified follow-up period.

Castration does not necessarily resolve aggressive incompatibility among male rabbits in all cases. Raje et al. (1997) kept a group of five castrated male NZW rabbits in an enclosure furnished with corncobs and cornhusk bedding and PVC pipes. Six days after the establishment of the group, a major conflict resulted in all but one rabbit suffering some kind of minor injury. The group was split into a pair and a trio. It is not made clear if this intervention was a long-term solution of the aggression problem.

### 3.1.4. Summary and Discussion

**Confined rodents and rabbits have no choice but to spend all of their time on the floor of their primary**

**enclosure.** Appropriate flooring is, therefore, an essential condition for their general well-being. A 1999 survey of 12 United States-based pharmaceutical and contract toxicology laboratories showed that more than 80 percent of the rodents used were housed in wire-bottom cages (Stark, 2001).

Wire-bottom caging is preferred by humans over solid-bottom caging because of the lower labor and monetary investment, but not by the animals who are at a high risk of developing pressure neuropathies and foot lesions on wire mesh floors (Fullerton and Gilliatt, 1965; Grover-Johnson and Spencer, 1981; Kraus, 1994; Kesel, 1995; Peace and Singer, 2001; Dimeo and Mitchell, 2005). When they have a choice, rodents will avoid wire mesh and make considerable efforts to get access to a solid floor.

From an economical standpoint solid-bottom caging is at a disadvantage as it requires absorbent litter for sanitary reasons. The caged animals themselves, however, prefer a bedded floor over a bare solid floor, probably for comfort and thermoregulation. They also show a markedly higher stress level—as reflected in elevated blood pressure and elevated heart rate—when they have to spend all the time on a wire mesh floor or a bare solid floor rather than on a bedded solid floor. This has been demonstrated in rats, and there is good reason to assume that it also applies to mice, guinea pigs, hamsters, gerbils and rabbits. It indicates that the provision of bedding material not only enhances animal welfare but also scientific methodology by minimizing stress.

Rabbits and most rodents prefer large fibrous bedding material such as straw, and shredded paper over sawdust. Gerbils prefer sand, which is their natural digging substrate (Ågren et al., 1989) and allows them to groom their fur in a species-typical manner (Pettijohn and Barkes, 1978).

**It is characteristic of rodents—but not of domesticated rabbits—to be afraid of crossing an area that offers no cover or hiding place from a potential predator.** The “open-field test” makes use of this disposition to assess a subject’s level of anxiety and fear. Living quarters that are essentially permanent open fields are, therefore, not conducive to the confined subjects’ well-being. A shelter not only provides protection, but can also be used as a relatively insulated nest box for resting, sleeping and the raising of pups, especially in conjunction with nesting material. If they are able to choose, rats, mice, gerbils and presumably also hamsters and guinea pigs prefer living quarters furnished with a shelter, or with a substrate that

can be used to build a shelter, over unprotected, barren living quarters of the same size.

For rats the shelter should be dark and rectangular, made of solid material with all corners enclosed, and have one or two access holes. Such a structure simulates a burrow, and rats will spend most of their time in it (Boice, 1977). Soft paper strips are the preferred nesting material for them. For mice the shelter should be a rectangular, roofless, narrow frame with perforated walls, or even better a paper-based enclosed structure with one entrance. Mice have a very strong urge to construct their own nests. Soft paper nesting material is, therefore, more important to them than a pre-formed shelter.

Rats, mice and hamsters readily work for appropriate nesting material (Jansen, 1969; Oley and Slotnick, 1970; Roper, 1975; Collier et al. 1990; Manser et al., 1998b), indicating that it is an essential resource with which they should always be supplied.

Gerbils and hamsters, and presumably guinea pigs as well also need dark covered shelters. It is not clear whether non-breeding rabbits take advantage of enclosed shelters. A shelf may be preferable, because it offers a covered refuge and elevated resting site without reducing the floor area of the cage.

**Housing rodents with same-sex companions in pairs or small groups rather than alone has several important benefits for the individual animal:**

- a. The biologically inherent need for social contact is met.
- b. The presence of compatible conspecifics buffers stress during fear-provoking situations.
- c. Interacting with other conspecifics helps in coping with boredom.
- d. Sharing a relatively large living area with social partners offers substantially more space for species-typical locomotion and exercise than the smaller single-animal cage.
- e. Uncontrolled breeding is not possible.

The assumption that certain regulatory authorities “prefer” single-housing should be challenged. Social-housing should become the norm whenever animals are compatible, and anything less has to be justified on the basis of sound science (Dean, 1999). Companionship is particularly important to female rats, who should never be caged alone.

Even male mice and hamsters, notorious for their aggressive propensities, prefer the company of a cagemate to being alone. This does not mean that they should always be kept in a social setting regardless of circumstances. In groups of male mice, there is usually only one animal who instigates fights. The removal of such an individual can often safeguard the integrity of the remaining group. The management of aggression in hamster groups has not yet been addressed in the scientific literature.

If they are not used for breeding, male guinea pigs are best housed in pairs. This eliminates social stress and the risk of injuries resulting from group-housing.

**Injurious aggression can make the social housing of mature rabbits a challenge.** It has been shown in pair- and group-housed nonhuman primates that the option of visual seclusion reduces aggressive tension while simultaneously promoting affiliative behaviors (Reinhardt and Reinhardt, 1991; McCormack and Megna, 2001). Does have a strong affinity to each other, but they also want to have the option of being alone at times. It is very likely that they would also benefit from sight barriers that allow companions to break visual contact with each other as needed, thereby avoiding aggressive tensions and fostering their long-term compatibility. Bucks are unlikely to tolerate each other. Single-housing is the safest, albeit not best, option for them.

Whenever it is deemed advisable to isolate rodents and rabbits for incompatibility reasons, it is an imperative to house the individual animal, including male rabbits (Batchelor, 1991), in an arrangement that allows him or her to at least see, hear and smell other conspecifics.

## 3.2. Environmental Enrichment

Environmental Enrichment is the provision of stimuli that promote the expression of species-appropriate behavioral and mental activities in an understimulating environment (Reinhardt and Reinhardt, 2002). Some species-typical behaviors, such as injurious aggression, are inappropriate within the context of confinement and are, therefore, excluded from this definition.

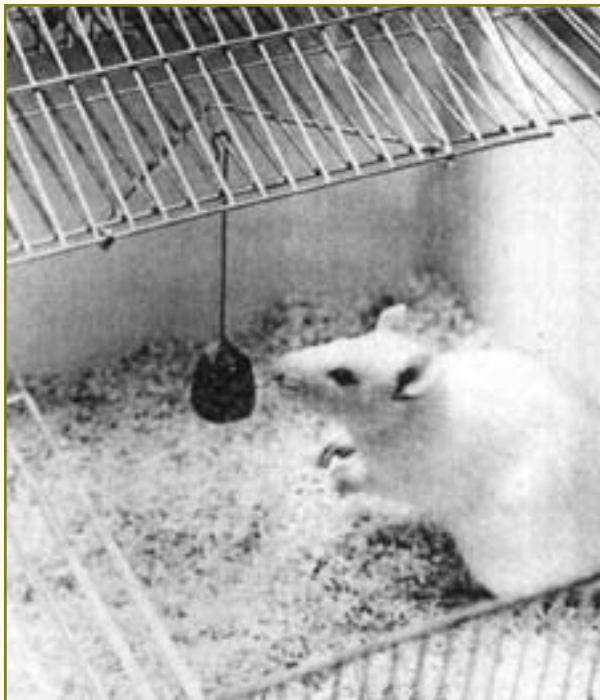
### 3.2.1. Objects and Structures

In the wild, rodents shun open areas, because they offer no visual protection from potential predators. In captivity, they will not use the space of an enclosure containing no structures evenly, but will shy away from the center and

spend most of their time at the periphery (Fredericson, 1953; Ross et al., 1966; White et al., 1989; Anzaldo et al., 1994), moving and resting close to the surrounding walls (thigmotaxis). The central, unprotected area of such an enclosure is functionally of little use to the animals.

Anzaldo et al. (1995) compared the preference of male Sprague-Dawley **rats** kept in groups of six for a barren standard cage versus a 50 percent smaller cage that was structured with two L-shaped partitions, subdividing the cage into a rectangular corridor with two entrances. These structures provided the rats with additional wall contact and a relatively small wall-encircled central area in which they could huddle and sleep together. During a 24-hour videorecording the animals spent 80 percent of the time in the smaller, yet structured cage and only 20 percent of the time in the larger, yet barren cage.

When the rats could choose between the small cage with the L-shaped partitions and another cage of the same size but furnished with two elevated platforms of unspecified height, they again preferred the cage with the increased wall contact options (Anzaldo et al., 1994). Wall contact is probably essential to the rats' sense of security, and hence is more important for them than platforms or



**Figure 16. A hanging piece of wood receives much attention by a caged rat.**

additional empty space.

Chmiel and Noonan (1996) gave singly caged Long-Evans rats the choice of staying in either half of a partitioned double-cage, one side of which was barren and the other furnished with one of several potentially enriching objects. The animals showed a significant preference for the furnished cage section when it was provisioned with a small birch ball (4.5 cm diameter), a small gnawing block (2.5 x 2.5 x 2.5 cm) drilled with two holes or a golf ball (4.5 cm diameter). The rats chewed all three objects into small bits over the course of four days. The small birch ball was the most attractive object. The rats showed no special interest in the furnished section of the cage when it contained a larger birch ball (7.6 cm diameter), a larger wood block (9 x 9 x 2 cm), a Y-shaped PVC section (6 cm diameter), a large or a small tin can (10.4 cm or 7.7 cm diameter), a small acrylic ball (2.5 cm diameter), a small acrylic block (1.6 x 1.6 x 1.6 cm), or a bone-shaped rawhide (7 cm long). These items had no enrichment value for them.

Eskola et al. (1999) videotaped groups of four Wistar rats who were each provisioned with one 6 x 6 x 6-cm aspen block with penetrating drilled holes (diameter of 1.9 cm) on each side and one 20 x 12 x 12-cm aspen box. The two objects were replaced once a week. Throughout a test period of five weeks both items were consistently used for gnawing, as measured in the reduction of wood volume. During the day, the rats spent about three percent of the time in contact with the block, 87 percent of the time *in* the box, and two percent of the time on top of the box. During the night, they spent 11 percent of the time in contact with the block, only 24 percent *in* the box, but 34 percent on top of the box. This activity pattern reflects the animals' nocturnal life style, with the dark shelter serving as a refuge and place to sleep during the day, and as an orientation platform during the night.

When given a choice between the large perforated aspen block and a much smaller (1 x 1 x 5 cm) non-perforated aspen block, the rats contacted the large one about seven percent of the time, and the small one less than one percent of the time (Mering, 2000).

Aspen wood is safe for the animals. Robertson (1999) exposed 20 Sprague-Dawley rats to 13.5 x 4 x 4-cm aspen sticks over a three-week period. The animals gnawed the wood but no signs of gastrointestinal tract injuries were found, suggesting that the material was safe.

Orok-Edem and Key (1994) tested two enrichment objects in groups of five Lewis rats. Each group was

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Novo Nordisk A/S, Denmark

**Figure 17. Rats prefer a cage furnished with a platform over an empty cage.**

exposed for a period of five days to (a) one approximately 16 x 2 x 0.2-cm tongue depressor made of birch wood, and (b) one 3-cm-long broom handle section hanging on a stainless steel clip from the top of the cage (Figure 16). Both objects triggered the primary behavior of gnawing. The suspended piece of wood received significantly more attention than the loose piece of wood, probably because it had the dynamic element of swinging back and forth when contacted. Nonetheless, the tongue depressor had to be replaced every day, because the rats shredded the material into tiny pieces within 24 hours. The suspended broom handle section remained in the cage throughout the test period, but there was hardly any wood left by the end of the study.

Patterson-Kane et al. (2001) gave singly caged Hooded Norway rats the choice of spending time in a barren cage versus an equally-sized cage furnished with either a PVC tube (8-cm diameter), a wooden platform (unspecified height), two gnawing sticks (~ 1-cm diameter), a plastic cylinder (15-cm diameter), or three tunnels glued together in a pyramid. The rats had a significant preference only for the cage with a platform (Figure 17). They had no apparent interest in the other objects. Nelson et al. (2003) videotaped singly caged Wistar rats in a test cage with two platforms of unspecified material that were placed at different unspecified heights. Individuals spent an average of 21 percent of one 24-hour recording session on the platforms. This could reflect the novelty effect of the platforms, because they were not the habitual furniture of the rats' home cages.

Collier et al. (1990) noted that singly caged Sprague-Dawley rats spent about seven percent of the 24-hour day in a freely accessible running wheel to which the animals had been habituated for 10 days. They readily worked by pressing a bar to obtain access to the wheel, indicating that wheel running was a rewarding experience for them.

Coviello-McLaughlin and Starr (1997) examined the effect of environmental enrichment—in the form of nestlets and cardboard tubes—on post-surgical premature wound clip removal by BALB/c nude mice. The percentage of animals removing wound clips dropped from 50 percent (6 of 12) in control animals kept in barren cages to 13 percent (4 of 30) in animals kept in enriched cages.

Hobbs et al. (1997) furnished the group-cages of four male CD-1, DBA/2 and B6CBF1 mice with two halves of a 10-cm-long plastic tube, a marble with a 1.3-cm diameter, and a nestlet. Six-hour videotape recordings during the night revealed that all three strains spent significantly more time contacting the nestlets (75 percent) than the tube (14 percent) and the marble (one percent).

Mice climb on and explore tubes, but there is no scientific report demonstrating their long-term usefulness



Maureen Hargaden, Hoffmann-La Roche Inc., Nutley, NJ, USA. ©Roche Laboratories Inc., 2005

**Figures 18a & b. Mice exploring ceramic and plastic tubes.**



Maureen Hargaden, Hoffmann-La Roche Inc., Nutley, NJ, USA. ©Roche Laboratories Inc., 2005

as burrows or sleeping sites (Figure 18a & b).

Sherwin (2004a) tested C57BL/6 mice individually in a preference cage with a mirror placed in one of the two interconnected cage sections. The animals showed no preference for the section with the mirror, but 13 of 16 mice spent less time in the cage section with the mirror than in the section without the mirror. Food consumption from the feeder immediately adjacent to the mirror was significantly lower than from the feeder in the other cage section. This indicates that the mirror had aversive qualities and was not a species-appropriate environmental enrichment object.

Sherwin and Nicol (1996) and Sherwin (1998) demonstrated in a series of experiments that TO mice are highly motivated to gain access to a metal rung-type running wheel with a 15-cm diameter. Harri et al. (1999)



Maureen Hergarden, Hoffmann-La Roche Inc., Nutley, NJ, USA. ©Roche Laboratories Inc., 2005

**Figure 19. Running wheels provide very attractive cage enrichment for mice.**

found that single C57BL/6 mice with free access to such a wheel spent approximately eight percent of the 24-hour day running (Figure 19).

Banjanin and Mrosovsky (2000) gave C57BL/6 mice the choice of using different types of running wheels. The animals showed a strong preference for a standard wheel that had a black plastic mesh (3.5 x 3.5 mm) wrapped around the outside of the rods.

Arnold and Westbrook (1997/98) placed a T-shaped PVC pipe (8-cm diameter) or a pint-sized clear glass jar (12.7-cm diameter) into the cages of groups of four same-sex golden hamsters. After being exposed to each of these two objects for two weeks, the hamsters contacted the jar more than twice as often as the pipe. The jar was primarily used for standing on top of it while peering out from the cage.

Hamsters are attracted to running wheels, spending much of their time exercising in them (Richards, 1966; Mrosovsky et al., 1998). Gebhart-Henrich et al. (2005) compared the behavior of female hamsters who had access to a functional or a non-functional large running wheel. Hamsters with a functional wheel showed significantly less stereotypical bar-mouthing than hamsters with non-functional wheels.

Reebs and St-Onge (2005) found that the animals have a strong preference for wheels that are larger than those often used for rats (35 cm vs. 23 cm diameter) and for completely circular wheels over truncated ones (Figure 20).

There are no data-supported published reports on environmental enrichment options for gerbils. Like other small rodents, gerbils spontaneously make use of running wheels (Roper, 1976).

Environmental enrichment options for guinea pigs have been described but their usefulness has not been tested and documented in the literature. Scharmman (1991) demonstrated with a photo that guinea pigs use gnawing sticks of unspecified dimensions.

Whary et al. (1993) provisioned the floor pen of eight female NZW rabbits with a 30 x 150-cm shelf mounted 25 cm off the floor, a 60 x 20-cm container filled with absorbent chips, and a 75-cm-long PVC tube (30-cm diameter) anchored to the floor in the center of the pen. Over a five-week period, the rabbits were seen near or in the litterbox 42 percent of the time, near or in the tube 45 percent, on the resting board ten percent and under the resting board three percent of the time. The litterbox was preferentially used

as a resting area and for playfully hopping in and out of the box in rapid succession. The PVC tube was very popular as a retreat and as an object of investigation. Individuals often rested in the tube for several minutes before being “gently” displaced by another rabbit entering the tube from behind. The tube was rarely empty. The surface of the shelf was used only occasionally for resting. The area under it served mainly as refuge during alarming situations (Figure 21).

Huls et al. (1991) placed one 23 cm long gnawing stick fastened to the side of the cage, one wooden parrot toy suspended from the top of the cage, and one brass wire cat toy in random order over a period of five days into the cages of eight NZW does and collected behavioral data, for each object during five 5-minute observations, at 15-second intervals. The rabbits chewed the stick and nudged it with their heads during 94 percent of observation intervals, the wooden toy during 77 percent and the brass toy during 79 percent of observation intervals. The long-term attractiveness of these three objects was not examined.

Wood items seem to be safe for rabbits. Brooks et al. (1993) noted no clinical problems in 48 animals who had access to regularly replaced fir sticks (12 cm long, 2.5 cm in diameter) throughout a two-year test period.

Johnson et al. (2003) tested a stainless steel rattle in singly caged NZW rabbits. The toy was contacted frequently in the beginning, but both females and males quickly lost interest and virtually ignored it after eight weeks. Harris et al. (2001) exposed singly caged NZW rabbits to three toys and videotaped the animals daily for one hour during a 15-day test period. The 10-cm-diameter polyethylene ball containing a stainless-steel jingle and the 11 cm long rubber toy generated much interest in the beginning, but by day six neither toy induced more than one minute of interaction both by females and males. The animals never paid much attention to the 10-cm-diameter nylon ball (Figure 22).

Enrichment objects made from plastic material can carry a certain risk. Shomer et al. (2001) reported an injury caused by a perforated hollow ball made of hard plastic. The ball had been considered safe, because it was made of non-toxic material, had no sharp edges, was too large to be swallowed or inhaled, and was judged too sturdy to be broken. However, the ball became lodged in the incisors of a doe, preventing her from eating or drinking, and causing trauma to her gums.

Edgar (2004) attached a mirror to the inside of the cages of singly housed NZW rabbits and videotaped the



**Figure 20. Hamsters seemingly enjoy running wheels, preferably large ones.**

individual subjects' behavior and location in the cage during a 7-day test period. No evidence was found that either does or bucks were attracted by the mirrors, even though their responses suggested that the animals did perceive the image in the mirror as a social counterpart (Figure 3). Jones and Phillips (2005) recorded the behavior of singly caged



**Figure 21. Rabbits use platforms primarily as a place of refuge during alarming situations.**

Tamara Godbey, University of British Columbia, Vancouver, Canada



Michelle Walsh, Cleveland Clinic Foundation, Cleveland, USA

**Figure 22. Rabbits quickly lose interest in plastic toys.**

Dwarf Lop and Lionhead does after mirrors were installed at the rear and both side walls of their living quarters. The rabbits spent an average of about 63 percent of the first hour sniffing and scrabbling at the mirrors versus about eight percent of the time on day seven. This indicates that the novelty effect of the mirrors was of short duration.

### 3.2.2. Space

Patterson-Kane (2002) tested female and male Wistar **rats** in a T-maze preference apparatus and found that all subjects showed a significant preference for a large (1620 cm<sup>2</sup>), yet barren cage over a small (540 cm<sup>2</sup>) barren cage. The preference was comparable to that shown by rats for a nest box vs. an empty cage (Patterson-Kane et al., 2001). Using the same paradigm, Patterson-Kane (2001) failed to detect, either in female or in male Hooded Norway rats, a significant preference for a large empty cage (1800 cm<sup>2</sup>) over a small empty cage (900 cm<sup>2</sup>). The animals were not inclined to work to get access to a barren cage that was twice as large as their home cage (Patterson-Kane et al., 2002).

Galef and Durlach (1993) gave male Long-Evans and Sprague-Dawley rats the choice to enter 1240-cm<sup>2</sup> barren cages that were 16.8 cm or 23 cm high. The rats exhibited no preference for either of the two cages.

Von Weiss and Taylor (1985) examined the issue of preferred cage height more systematically in male Wistar rats. The subjects had simultaneous access to barren standard cages (840-cm<sup>2</sup>) that were either lower or higher than the legally required 15 cm. The rats showed a

statistically significant preference for the 18-cm-high cage. The percentage of a 48-hour test period spent in the four cages was:

- 10-cm-high cage: 7 percent
- 14-cm-high cage: 16 percent
- 18-cm-high cage: 43 percent
- 22-cm-high cage: 16 percent

Sherwin and Nicol (1997) trained male TO **mice** to operate a switch to move from a 270-cm<sup>2</sup> barren cage to another barren test cage that was either smaller (196 cm<sup>2</sup>), moderately (360 cm<sup>2</sup> and 625 cm<sup>2</sup>), or substantially larger (1600 cm<sup>2</sup>). The animals readily worked to gain access to the test cages—probably out of curiosity—but failed to show a preference for a larger vs. smaller cage. The similarity in responses is noteworthy, since the smallest cage provided less space than the standard cage—just enough space for the mouse to turn around—while the largest cage offered several times more space than the standard cage. Sherwin (2004b) repeated this study with group-housed female CB57 mice using correspondingly-sized cages and obtained equivalent results: The mice did not differentiate between small and large barren cages in their motivation to get access to them, probably because none of the cages offered structured space to be explored and functionally used.

Mahon et al. (2005) determined that breeding trios of C57BL/6 mice had a higher birth rate (9.6 pups/female) in presumably unfurnished, larger than normal 20 x 43 cm cages than in standard 15 x 25 cm cages (7.2 pups/female). Keeping the mice in larger cages not only improved their breeding performance but it also decreased labor investment.

Krohn et al. (1999) compared the activity and behavioral expressions of individually housed female Ssc: CPH **rabbits** kept in barren cages that were 2800 cm<sup>2</sup> or 5600 cm<sup>2</sup> and found no significant differences.

### 3.2.3. Feeding Enrichment

Rodents and rabbits are biologically adapted to spend a great portion of their time searching for and processing food. Neuringer (1969) and Carder and Berkowitz (1970) showed in rats that this inherent foraging drive is so strong that the animals readily work (press a lever) for food even if identical food is freely available to them. This behavioral response is so conspicuous that it has

received a special technical term, “contrafreeloading” (Inglis et al., 1997).

Wrightson and Dickson (1999) redesigned the traditional food hopper for group-housed **rats** of unspecified strain by covering the access area with a sheet of aluminum, leaving only a narrow slot open. Rather than rapidly collecting and eating their pellet ration, the rats now had to skillfully retrieve their food, thereby engaging in a more natural foraging-like behavior. After eight months of exposure to such a limited-access hopper, the rats were still slim (prevention of obesity!) and showed no adverse clinical effects. Since up to three rats could obtain food at the same time, the new hopper did not lead to antagonism resulting from competition. Johnson et al. (2004) promoted more foraging activities in pair-housed Wistar rats by placing their daily pellet ration in a 3-cm raised metal dish and then covering the food with gravel. This simple modification of food presentation increased significantly the time that the rats spent obtaining their food.

There are no published articles addressing feeding enrichment possibilities for **mice**, **hamsters** and **gerbils**.

Sutherland and Festing (1987) concluded from casual observations that **guinea pigs** need hay, and that outbreaks of pathological hair-pulling and -chewing may occur when hay is not provided. Guinea pigs seem to indicate the great pleasure they derive from burrowing and foraging in hay by vocalizing when attending personnel are about to replenish it (Figure 23).

Metz (1987) gave groups of five NZW **rabbits** continual access to straw over a period of four weeks. The animals spent an average of 11 percent of the 24-hour day exploring, nibbling and eating straw. Lehmann (1990) mounted a rack of hay in the pen of ten NZW rabbits and replenished it daily. Individuals spent eight percent of the time retrieving and eating hay (Figure 24). Engagement with hay was highest at dawn and dusk. Berthelsen and Hansen (1999) distributed hay on the cage tops of singly housed NZW x French Lop rabbits. The animals retrieved and ate hay 16 percent of the time during the day and nine percent of the time during the night.

Lidfors (1997) provisioned singly caged NZW rabbits daily with (a) either 20 g of hay stuffed into a plastic bottle so that the subjects had to manipulate the material to retrieve it, or (b) two aspen sticks of unspecified dimensions. Over a four-week observation period, hay was contacted 11 times more often than the sticks.



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**Figure 23. Old rabbit cages can be recycled for group-housed guinea pigs. The Macrolon IV cage provides an area of solid flooring with a bedding of sawdust and daily hay. The guinea pigs defecate and urinate primarily on the grid floor of the rabbit cage, but they spend most of the time in the Macrolon cage nibbling and eating hay, sleeping in hay and hiding in “dens” made of hay.**



Novo Nordisk A/S, Denmark

**Figure 24. Offering hay and vegetables in a hay rack, promotes foraging behaviors in rabbits.**

### 3.2.4. Interaction with Humans

Werner and Latané (1974) and Werner and Anderson (1976) noticed that male and female Sprague-Dawley **rats** readily get attracted to the human hand that interacts with them in rat fashion by poking, lifting, rubbing and scratching, tapping, and tumbling but, of course, never holding them. Davis and Pérusse (1988) confirmed in female and male Wistar rats, that about half of the subjects tested worked in a Skinner box for petting by and social interaction with a familiar human in the absence of any other reward. Positive interaction with humans can, therefore, serve as species-appropriate environmental enrichment for these rodents.

Hirsjärvi and Junnila (1988) and Hirsjärvi and Valiaho (1995) exposed adult male Wistar rats, who had been either (a) gently handled on a regular basis or (b) left with the routine care involving minimal handling, to the presence of a potential predator—the experimenter—standing next to an open test arena. The gentled rats showed lower frequencies and durations of freezing and significantly lower incidences of loose stool and rigid movements than the nongentled rats, suggesting that the regular gentle handling had reduced their fear towards the experimenter (Figure 25).



Greg Morton/Media Group, University of British Columbia, Canada

**Figure 25. Through regular friendly visits, the investigator can establish a relationship with her/his rats that is not preconditioned by fear. This will minimize stress reactions during procedures that require the handling of the animals.**

Shyu et al. (1987) compared the pharmacokinetics of two different drugs (amikacin and ticarcillin) injected in Sprague-Dawley rats who were either (a) handled daily prior to the study and then held—rather than wrapped in a towel—and petted by the investigator during the experimental procedure or (b) were not extra handled but simply picked up and wrapped in a towel while the experimenter obtained a tail vein blood sample. For rats, who were handled in a manner to minimize stress, pharmacokinetic profiles of the two drugs were typical. Aberrant serum concentration-time curves were observed in rats who received no extra gentle handling.

Nerem et al. (1980) tried to alleviate the potential stress associated with single-caging in male NZW **rabbits**. The animals were assigned to an atherosclerosis study. One group of animals received normal laboratory animal care while the other group received special attention by one person who talked to, played with and stroked each subject daily during brief visits (Figure 26). The rabbits with human contact were significantly less susceptible to atherogenesis—as measured in aortic sudanophilia—than control rabbits. The study was repeated and the result confirmed with a different set of rabbits.

### 3.2.5. Summary and Discussion

**New things elicit curiosity, and if they are not dangerous, exploration. Beyond this novelty effect, however, many objects quickly lose their attraction and hence have no true environmental enrichment value for the confined animal.** It is sometimes recommended to rotate toys in order to recharge their novelty effect. This strategy may not be practicable in facilities that keep thousands of animals for research purposes. Rather than investing the extra time needed to exchange the toys on a regular basis, it would probably be more effective and less expensive to offer the animals enrichment in which they do not lose interest over time.

Toys do have their value under the condition that their long-term effectiveness as enrichment gadgets has been tested and documented for the species they are designed for.

Properly-sized wooden objects prompt rats to engage in species-typical gnawing and manipulating without causing noticeable clinical risks. Along with the natural dehydration process of the wood, these behaviors make the object constantly change its configuration, size and texture. The gnawing block or gnawing stick thereby becomes a



Natasia Down, York University, Toronto, Canada

**Figure 26. The affectionate relationship of attending care personnel with the animals in their charge is a safeguard not only that the animals receive optimal care, but also that they are not unduly disturbed by the presence of people during scientific experiments.**

dynamic object that, unlike indestructible objects, maintains its novelty effect until it is completely worn down. Rats don't seem to get bored by wooden material but by plastic or other relatively indestructible objects. They should, therefore, always have access to gadgets made of natural wood to help them cope with boredom.

Objects that can be turned into nesting material seem to be the preferred environmental enrichment for mice.

With the exception of guinea pigs, rodents make use of running wheels with consistency. They should, therefore, be provisioned with appropriate running wheels to allow them to engage in some exercise.

**Vertical structures offering additional wall contact and elevated look-out posts provide particularly suitable environmental enrichment for rats.**

PVC tubes are very useful enrichment objects for rabbits. Instead of commercial toys in which the animals quickly lose interest, each rabbit enclosure should be furnished with one or several PVC tubes as objects for investigation and places for retreat.

**Space has little or no enrichment value unless it is structured.** Confined rats, mice and rabbits make no clear distinction between a barren cage that is small and one that is large and has the same shape. This does not imply that they would not benefit from cages larger than the minimum-size; the additional space of larger cages should serve to hold appropriate environmental objects and structures that promote species-typical behaviors.

Since rodents shun open areas, the determination of their minimum floor space requirements will also have to take the shape of the cage into consideration. If a cage is not furnished with a shelter, the cage should be relatively long and narrow. This will minimize the area of the central open field that is avoided by the animals.

**Systematic preference studies are required to objectively determine the most species-adequate shape of the primary enclosure and the minimum horizontal and vertical unstructured space requirements of rodents and rabbits.** The pilot study by von Weiss and Taylor (1985) demonstrates for male Wistar rats that the animals have a strong preference for a cage that is moderately (3 cm) but not substantially (7 cm) higher than the legal requirement of 15 cm. The legal determination of minimum horizontal space is particularly important for caged rabbits (Figure 27a & b). These animals are prone to develop muscular atrophies, poor bone growth and backbone distortions as a result of lack of locomotor activity in small cages (Wieser, 1984; Bigler and Lehmann, 1991; Rothfritz et al., 1992) that may provide the rabbit the legally required floor space to make normal postural adjustments (United States Department of Agriculture, 2002) but is not large enough to allow for the rabbit-typical hopping.

**Modifying the food hopper so that skillful manipulations are required to obtain the standard food ration is a simple, practical and effective enrichment technique** allowing rats, and probably also mice, hamsters and gerbils, to get more actively involved in the feeding process. The idea of having the animals work for their daily food ratio, and thus engage in foraging behavior by modifying their food boxes, has been applied with success also in nonhuman primates (Reinhardt, 1993; Murchison, 1994). It is probably the least expensive, yet most effective feeding enrichment option.

Guinea pigs and rabbits are easily prompted to engage in foraging behavior by offering them hay on a daily basis.

**There is a need to study the impact of regular positive interaction with humans, and to implement the findings in the daily work schedule of attending care personnel and animal technicians.** The few articles dealing with this issue in rats and rabbits strongly suggest that the confined animal subject receives special benefits from such interaction not only in terms of distraction in an otherwise boring environment but also in terms of stress reduction and decreased fear of humans.



**Figures 27a & b. The dimensions of the primary enclosure have to take species-typical postures into account to provide reasonably comfortable living quarters. For an adult, 4-6 kg NZW rabbit the enclosure should be no less than 80 cm long to allow for resting in the rabbit-typical lateral sternal position (Figure 27a) and 75 cm high for sitting in the rabbit-typical lookout position (Figure 27b).**



Gunn-Dore, 1997

Developing a relationship with rodents and rabbits that is based on trust rather than fear will also provide the condition for positive reinforcement training, an area that is being explored intensively with great success in nonhuman primates assigned to biomedical research (Reinhardt, 1997), but that has been largely ignored by researchers working with rodents and rabbits.

## 4. OUTLOOK

Inertia of tradition is a big hindrance for concerned animal care personnel, veterinarians and young scientists who wish to change the *status quo* of rodents and rabbits assigned for biomedical research. The outlook for animals kept in European facilities is more promising than for those kept in the United States. While European animal welfare law (European Economic Community, 1986) covers *all* rodents, including those who make up the bulk of laboratory animals, i.e., rats and mice, US “animal” welfare law and its regulations explicitly *exclude* without any stated reason these animals in its definition of the term *animal* (Animal Welfare Act, 2002; United States Department of Agriculture, 2002).

This lack of protection makes the situation for rats and mice particularly problematic in the United States, because there is no serious legal incentive to take the welfare of these “non-animals” into earnest consideration as we design their living quarters and define the ways they are handled during procedures. Fortunately, many animal caretakers, animal technicians and investigators who do the hands-on work with rats and mice and have daily contact with their charges *do* regard rats and mice as true animals and treat them accordingly, by making their lives as comfortable and bearable as possible (Figures 28-30).

The combined number of rodents and rabbits used for biomedical research in the United States and Europe is



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**Figure 28.** A hammock increases the usable cage space for mice.



Maureen Haigden, Hoffmann-La Roche Inc., Nutley, NJ, USA. ©Roche Laboratories Inc., 2005

**Figure 29.** Nesting material allows mice to build their own comfortable quarters.

>29,000,000. Of these animals, an estimated 70 percent (>20 million) are kept in US laboratories and 30 percent (>9 million) in European laboratories (Gauthier, 2004; Kaliste, 2004).

Even though most of the rodents and rabbits are used in US research facilities less than half (47 percent; 123/260) of the relevant articles pertaining to the improvement of



**Figure 30.** Rats are sensitive animals who deserve to be treated with consideration of their well-being.

their housing and handling conditions were published in US journals; more than half (52 percent; 134/260) of the relevant articles were published in Europe.

The outlook for rodents and rabbits kept in research facilities will be more promising in Europe until researchers in the United States seriously acknowledge the fact that improving the living conditions of their animals is a prerequisite for scientifically sound research methodology.

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The Animal Welfare Institute (AWI) is a non-profit charitable organization founded in 1951 to reduce the sum total of pain and fear inflicted on animals by humans. AWI encourages the refinement of housing and handling for animals in research and the development and use of non-animal alternatives.

Additional goals are:

- Preserving species threatened with extinction
- Prohibiting the use of steel-jaw leghold traps and other inhumane methods of wildlife control
- Reforming cruel production systems for the rearing of animals on farms
- Regulating humane transport conditions for all animals

Our policy on vertebrate animals in experimentation and testing is that they should only be used when there is no feasible alternative, following the review of a carefully designed experiment based on knowledge of existing literature. The smallest possible number of animals and most suitable species should be used, and they should be maintained in an optimum environment under the care of trained, sympathetic personnel. Pain, fear and anxiety should be prevented by judicious experimental design and generous use of anesthetic, analgesic and tranquilizing drugs. Finally, threatened species should only be used for experiments conforming to the requirements for human experimentation, and endangered species should never be used. AWI's full *Policy on the Use of Vertebrate Animals for Experimentation and Testing*, our databases and more information on the organization are available at [www.awionline.org](http://www.awionline.org).

