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Refinement and Reduction Outcomes of Cage Furniture and Restricted Feeding in Laboratory Rats

Doctoral dissertation

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Department of Biosciences
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University of Helsinki
ABSTRACT

The most recent European housing and care regulations for laboratory rats mandate provision of a structured environment and group housing. Dividing structures and shelters in the cage offer rats opportunities to seek or avoid contact with other group members and hence are regarded as beneficial to animal welfare.

In order to compare the physical environment of the IVC and the open cage, temperature, relative humidity, lighting and sound levels of the cages were measured. BN/RijHsd and F344/NHsd male rats were used, and they were housed in IVC- or open cages of the same type, three rats per cage, one of them carrying a telemetric transponder. Four groups and a crossover design were used: two groups with a maze made of crossing two aspen boards, a rectangular aspen tube group and controls. In one maze, drilled holes were loaded snugly with food pellets; rats had to gnaw wood to gain access to their food. Rats and food were weighed before and after each study period. The means of locomotor activity and means and coefficient of variations for mean arterial pressure (MAP) and heart rate (HR) were calculated for days 2, 6, 10 and 14 in each period. As a way of determining which of the statistically significant MAP and HR mean changes were biologically meaningful, the corresponding night-day differences of the controls were used in this two step assessment. On day 8 of each two week period, the rats were changed to clean cages and on day 11 exposed to IG-gavage. The means of activity, mean arterial pressure and heart rate were processed for the first hour subsequent to the procedure and thereafter separately in the light and dark periods and for the two cage types. Baseline values for each rat, for both dark and light and cage types were calculated from recordings made 24 h earlier; and these were subtracted from the corresponding response values.

In the study of the physical environment of the cage types, there were differences in all measured parameters. In F344 rats, diet board was more effective in controlling weight, but when combining the strains, all comparisons with diet board were significant. In both cages, the F344 rats were generally more active than the BN rats during the dark phase, but not during the light phase. In the IVCs, both board types lowered MAP of F344 rats throughout the two week period and at the end of that period. Plain board was found to be the better of the two; hence dividing walls with or without restricted feeding seem beneficial for the welfare of F344 rats. None of the MAP or HR differences in BN rats were biologically significant. The MAP CV results showed that cage furniture may be used to achieve a considerable reduction value in blood pressure studies, but the outcome is strain-specific. Neither of the strains exhibited any statistically significant differences in faecal corticosterone or IgA excretion to these items. Based on the MAP results, the tube appeared to be a poor choice for F344 rats, whereas for BN rats, all furniture items seemed beneficial, with both board types apparently superior to the tube. In general, F344 rats had higher faecal corticosterone levels than BN rats with the reverse being true for secretory IgA values.

In conclusion, LA and cardiovascular parameters seemed appropriate ways to evaluate the impact of cage furniture on physiological parameters, and covered structures such as tubes do not seem to provide any refinement value in these two rat strains.
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Niina Kemppinen
Helsinki, October 2009
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<td>2Rs</td>
<td>Reduction, Refinement</td>
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<tr>
<td>3Rs</td>
<td>Reduction, Refinement, Replacement</td>
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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<td>ANS</td>
<td>Autonomic nervous system</td>
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<td>BALB/c</td>
<td>Inbred mouse strain</td>
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<td>BN</td>
<td>Brown Norway – an inbred rat strain</td>
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<td>BPM</td>
<td>Beats per minute</td>
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<td>C57BL/6J</td>
<td>Inbred mouse strain</td>
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<td>CRF</td>
<td>Corticotrophin releasing factor</td>
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<td>CR</td>
<td>Caloric restriction</td>
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<td>CV</td>
<td>Coefficient of variation</td>
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<td>DBA/2</td>
<td>Inbred mouse strain</td>
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<td>DPB</td>
<td>Diastolic blood pressure</td>
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<td>EC</td>
<td>European Commission</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>F344</td>
<td>Fischer 344 – an inbred rat strain</td>
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<td>FP7</td>
<td>Seventh Framework Programme</td>
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<td>GR</td>
<td>Glucocorticoid receptor</td>
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<td>HEPA</td>
<td>High efficiency particulate air</td>
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<td>HPA</td>
<td>Hypothalamic pituitary adrenal</td>
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<td>HR</td>
<td>Heart rate</td>
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<td>IG</td>
<td>Intragastric</td>
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<td>IgA</td>
<td>Immunoglobulin A</td>
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<td>IVC</td>
<td>Individually ventilated cage</td>
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<td>LA</td>
<td>Locomotor activity</td>
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<td>MAP</td>
<td>Mean arterial pressure</td>
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<td>MR</td>
<td>Mineralocorticoid receptor</td>
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<td>PE</td>
<td>Point estimate</td>
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<td>PP</td>
<td>Pulse pressure</td>
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<td>ppm</td>
<td>Parts per million</td>
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<td>QTL</td>
<td>Quantitative trait locus</td>
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<td>RH</td>
<td>Relative humidity</td>
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<td>SBP</td>
<td>Systolic blood pressure</td>
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<td>SEM</td>
<td>Standard error of mean</td>
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<td>SNS</td>
<td>Sympathetic nervous system</td>
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<td>T&lt;sub&gt;b&lt;/sub&gt;</td>
<td>Body temperature</td>
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, referred to in the text by their chapter numbers.


CHAPTER I

GENERAL INTRODUCTION
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1.1 Alternative methods to the use of animals in research

It is realistic to state that laboratory animals will still be used in research in the near future (Festing 2004); indeed there are several indications that their use may even increase. For example in Europe, the European Commission (EC) intends to double funding for basic research (EU 2007a); this will undoubtedly have consequences on the quantity and quality of animals being used. Similar trends in funding can be seen elsewhere where governments and funding agencies place their trust and financial backing on science as a way to solve many global problems. Concomitantly, the use of genetically altered animals is increasing all over the world. In Europe, the new Chemicals directive (Registration, Evaluation, Authorisation and Restriction of Chemicals, REACH) is estimated to add 8-30 million animals to the numbers already being used. The latest statistics for 2002 and 2005 from the EC also supports this view; the number of animals used has increased from ten to 12 million in three years (EC 2005, EC 2007b).

The continuing need for the use of animals in research places an even greater emphasis on animal welfare and novel ways to measure it. Animal welfare refers to implementation and measurement of ways to verify efficiency adhered to the alternative methods. Contrary to the common belief, the principles behind the alternative definitions are not new. These principles were introduced already 1831 when British physiologist Marshall Hall proposed five principles that should govern animal experimentation (Paton 1984):

1. An experiment should never be performed if the necessary information could be obtained by observations.
2. No experiment should be performed without a clearly defined and obtainable objective.
3. Scientists should be well-informed about the work of their predecessors and peers in order to avoid unnecessary repetition of an experiment.
4. Justifiable experiments should be carried out with the least possible infliction of suffering (often through the use of lower, less sentient animals).
5. Every experiment should be performed under circumstances that would provide the clearest possible results, thereby diminishing the need for repetition of experiments.

Marshall Hall’s principles were translated to 3Rs by Russell and Burch in their 1959 book “The Principles of Humane Experimental Technique”. The concept of the 3Rs aims at replacing, reducing or the refining use of laboratory animals. According to them, “Replacement” means “the substitution for conscious living higher animals with insentient material”, “Reduction” means “reduction in the numbers of animal used to obtain information of a given amount and precision”, and “Refinement” means “any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used” (Russell & Burch 1959).

Today these 3R principles constitute the alternatives to the use of animals and have been incorporated into most regulatory documents and mentioned in a plethora of
guidelines and recommendations (Council of Europe 1986, Declaration of Bologna 1999, EC 2006) dealing with the use of animals in research and in funding of such activity, e.g. ethical rules in the Seventh Framework Programme (FP7) (EC 2007a).

It is increasingly evident that animal-based research is not always done in the best possible way. There is doubt with respect to the extent to which the refined methods developed in laboratory animal science are actually applied to studies using animals. Richardson & Flecknell (2005) found that postoperative pain control was used in less than 20% of potentially painful research procedures; Olsson et al. (2007) discovered only a few references to refinement in studies using neurodegenerative rodent models. Furthermore, recent systematic reviews raise questions over the benefit of preclinical research on animals for the development of clinical applications, on the grounds of inappropriate design and methodology (Dirnagl 2006) or incorrect timing (Pound et al. 2004) of animal-based research in relation to the clinical studies.

While the Replacement is the ultimate objective, complete avoidance of the use of sentient beings does not, unfortunately, appear to be possible (Festing 2004). Until this ultimate objective has been achieved, animals will continue to be used in those situations where no Replacement is available. It is considered morally wrong and even cruel not to extend the principles of Reduction and Refinement (the 2Rs) to those animals during the meantime.

Overly large variation of result parameters is undesirable in research because it is bound to increase the number animals needed in a study, that is against the desired aims of reduction (Festing et al. 2002). Refinement includes methods that are designed to improve housing or procedures of the animals. The scientists quite correctly expect reliable results and here both refinement and reduction have an essential role to play. It is not only the improvements in welfare but also a uniform nature of welfare that are important both to the scientists and the animals.

In 2005, COST (European Cooperation in Science and Technology) Action B24 submitted the 2Rs Initiative to the FP7. In this Initiative, it was acknowledged that although Replacement is the ultimate objective - i.e. to completely avoid the use of sentient beings - unfortunately this is not yet possible. Until this objective has been achieved, animals will continue to be used where no replacement is available. The inclusion of 2Rs funding into the FP7 was supported by 50 International and European universities, research institutes, scientific associations and animal welfare organizations, but implementation of measures has not taken place.

Refinement and Reduction alternatives are interrelated; studies may do well in refinement while major compromises occur in reduction or the opposite (Nevalainen 2004). The optimum and the prohibited directions are clear but in the remaining choice combinations one has to make a value judgement on whether refinement or reduction is more important. In Figure 1.1, the relationship of the 2Rs is illustrated with two perpendicular axes; in addition to fewer animals and refined welfare, we should aim at better science resulting from animal use.
Figure 1.1 Reduction (x-axis) and Refinement (y-axis) dimensions for assessment of any care routine or study procedure on animals. Arrows point to a direction of less harm to the animals, and to a desired concomitant change in research quality, both essential elements to be addressed in animal studies.

Russell and Burch (1959) also argued that application of the 3Rs should not be detrimental to the scientific outcome of animal studies. This aspect should also be addressed by systematic research, with the aim of establishing which of the 3R alternatives results in better science and even the opposite. It is quite clear that all of the 3R methods may not be free of adverse consequences on science, and those may be associated with consequential wastage of animals.

The implementation of the 3Rs can actually lead to improvements in scientific quality. A systematic scientific approach to find out which of the alternatives indeed results in better science is necessary. In other words, the scope of ethics in animal studies should expand to encompass scientific reasoning. The twin aims of ethical and scientific integrity can and indeed should be addressed with education based on targeted research focussed on the topic.

The Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes states that the Commission and the EU Member States must actively encourage and support the development, validation and acceptance of methods which could reduce, refine or replace...
the use of laboratory animals. This has not been fully implemented; and the ongoing revision of the Directive is anticipated to be more stringent on the requirement of implementing the alternative methods, i.e. the 3Rs (EU 2008).

Closer integration of laboratory animal science and research using animals is a necessity. Currently there is European research activity on implementation of the 3Rs in animal testing (European Centre for Validation of Alternative Methods (ECVAM), FP7), the main focus being on replacement; this covers only about 25 % of animal use and one of the 3Rs, e.g. excluding fundamental and applied research. The belief is that the establishment of scientifically proven 3R methodology combined with mandatory application will improve Europe’s competitiveness.

1.2 Animal welfare

The definition of animal welfare varies in many ways depending on the author’s point of view, and as Newberry (1995) has said, “the concept of animal welfare is a vague notion that evades precise definition and is used inconsistently in the literature”. Clark et al. (1997a) also considered the animal welfare a vague concept, which can neither be viewed in a purely objective manner nor simply described, defined, or assessed. Furthermore, Clark et al. (1997a) used the term “animal well-being” because it is more widely used in the United States rather than the alternative “animal welfare”.

The classical definition for animal welfare is an individual’s state as regards its attempts to cope with its environment (Broom 1986). Moreover, it has been stated that coping of the animal refers to both how much work has to be done in order to cope with the environment and the extent to which coping attempts are successful (Broom & Johnson 1993). In analogy to this coping definition, Webster (1995) has defined the welfare of the animal as “determined by its capacity to avoid suffering and sustain fitness”.

Fraser et al. (1997) listed three ethical concerns that reflect the quality of life of animals and can also be used as bases for animal welfare definitions. First, animals should lead natural lives through the development and use of their natural adaptations and capabilities, including natural behaviour. These authors suggested that the genetically encoded “nature” of an animal can be viewed as the set of adaptations that an animal possesses as a result of its evolutionary history, and the set of genetically encoded instructions that guide the animal’s normal development. Second, animals should feel well by being free from prolonged and intense fear, pain and other negative states, and by experiencing normal pleasures, i.e. animals have feelings. Third, animals should function well, in the sense that they experience satisfactory health, growth and normal functioning of physiological and behavioural systems. In animal welfare definitions, the biological functioning of animals is often linked to certain concepts such as fitness and stress.

All the three concerns can be seen in the “five freedoms” proposed by the Farm Animal Welfare Council of United Kingdom in 1993 (Webster 2003). Five freedoms categorise the different elements necessary for good welfare and husbandry provisions and their promotion. These freedoms:
1) freedom from thirst, hunger and malnutrition  
2) freedom from discomfort  
3) freedom from pain, injury and disease  
4) freedom to display most normal patterns of behaviour  
5) freedom from fear and distress  

Animal welfare has received considerable attention also in the EC legislation. In the Amsterdam Treaty (EU 1997), the protocol on protection and welfare of animals confirms the EC’s intension to ensure improved protection and respect for the welfare of animals as sentient beings. It also states that while formulating and implementing the EC’s agriculture, transport, internal market and research policies, the EC and the Member States shall pay full regard to the welfare requirements of animals.  

Since the animal welfare is a complex concept with many definitions, there are no simple methods to assess it. Different methods measure only different components of the welfare rather than the animal welfare itself (Rushen & de Passillé 1992). According to Clark et al. (1997b) classic and practical assessment of animal welfare includes a combination of animal appearance, performance, behaviour, productivity, disability, injury, disease, longevity, mortality and of the state of an animal’s environment. Thus it is essential to use a variety of welfare indicators if an adequate assessment of animal housing and management systems (Broom 1991).  

The animal welfare has been assessed e.g. with preference and behavioural tests, number of wounds, disease and reproductive levels, and adrenal activity (Broom 1988, 1991). Preference tests can show what animals choose and how hard they will work for a preferred event or object. Behavioural observations tell us whether the animals are able to carry out normal behaviour in their environment. Injuries, declined reproductive capabilities and high disease frequencies have been used as incidences of reduced fitness of animals. Adrenal activity responses are brief and the responses of the adrenal cortex decline after a few hours and thus they are usable for assessment of short-term welfare problems. However, if adverse conditions continue for many hours, also bursts of glucocorticoid production can be detected (Broom 1988).  

1.3 Individually Ventilated Caging (IVC)  
Until recently the prevailing housing system of laboratory rodents has been open, conventional cages. In these cages, animals are in direct contact with the ambient room air and thus also with the other animals in the room, which renders transmission of infectious agents, gas emissions, e.g. ammonia, and allergens, to room air and other cages. The development of pressurized individually ventilated housing system for laboratory rodents began already 1963 at the Jackson Laboratory in USA (Clough et al. 1995). During the last decade, the scientific community has witnessed the massive introduction of IVC-caging, where each individual cage receives its own HEPA filtered air flow, primarily designed for animal health status maintenance and occupational safety for the personnel.  

The IVCs have clear advantages over open cages; they provide protection against infections to animals, drastically decrease emissions from animals and cages to and compensate for poor ventilation in the room.
(Brandstetter et al. 2005). Even though the actual cages may be the same as those used in open cage systems, the physical environment inside is not (Clough et al. 1995, Teixeira et al. 2006).

Air to the IVC system can be supplied either from central ventilation or directly from the room, and the same applies to the exhaust air. The latter arrangement is typical to older facilities or those in transition from open cages to IVCs. This division has consequences on effective ventilation and other physical environmental characteristics inside the cage. Table 1.1 is a summary of studies on physical environment of IVC and resulting effects both on mouse and rat welfare.

The IVC systems are efficient in isolating the animals from other animals possibly harbouring animal pathogens because all incoming air is HEPA-filtered. It has been shown that the IVC system effectively prevents the transmission of particles from room to cage air and also between cages (Clough et al. 1995, Myers et al. 2003). Hence it is no wonder that IVC systems are gaining popularity despite the rather high investment costs associated with their purchase.

There are also clear occupational health benefits to the personnel associated to IVC equipment, i.e. a reduction in both the levels of airborne allergens and ammonia (Keller et al. 1983, Lipman et al. 1992 Lipman 1999, Renström et al. 2001, Teixeira et al. 2006). Airborne allergens with IVC caging appear to be about 1.5% of the levels applicable irrespective of the source of incoming air. Ammonia levels – if air is circulated from room air and back – depend on the ventilation efficacy of the room. A low ammonia content is important for both human and animal health; e.g. 5 ppm concentration in the cages leads to a weaker defence capability of the respiratory tract (Dalhamn 1956).

The air changes in the IVC cages can be considerably higher with the same or even lower air flow than in animal rooms simply because the combined cage volume is always much less than that of the room. This is considerable improvement compared to open cages, where the room ventilation rate of 5-20 changes per h reduced levels of CO₂, ammonia, relative humidity (RH) and temperature inside the mouse cage (Reeb et al. 1997). Common ventilation rates inside IVC-cage vary between 25-120 changes per h, but also extremely high, such as over 600 air changes per h, have been examined (Teixeira et al. 2006). Preference studies on both BALB/c mice and Sprague-Dawley (SD) rats showed preference to lower range of the common air change rates (Baumans et al. 2002, Krohn et al. 2003b). However, mice preferred cages with a somewhat higher air change if the cage was provided with a covered air supply and nesting material; this combination appeared to lower the stress effect associated to high ventilation rates (Baumans et al. 2002).

The high ventilation in IVCs results in better air quality, but has also a drying effect on bedding. The latter allows lower cage changing frequency in IVC than in the open cages. Different cage ventilation rates (30-100 changes per h) have been evaluated with three different cage changing intervals (7, 14 and 21 days) in C57BL/6J mice (Reeb et al. 1998, Reeb-Whitaker et al. 2001). Relative humidity and concentrations of ammonia and carbon dioxide (CO₂) were lower at higher
Table 1.1 Summary of mouse and rat studies on effects of the IVC-system.

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ventilation rates, whereas the lowest ventilation rate with weekly cage changing caused excessive pup mortality. Furthermore, plasma corticosterone levels in mice were lower when the cages were changed less frequently. A cage change every two weeks with a ventilation rate of 60 air changes per hour seemed to provide ideal conditions for mouse health and housing. The same applies to RH, CO$_2$ level and also to the lower temperature in rats housed in forced-air ventilated cages with high ventilation rates, this study concluded the optimum ventilation rate to be 60 air changes per h with rats as well (Hasegawa et al. 1997).

The IVC system is dependent on a continuous supply of electricity. If the power supply fails or the sealed cage is detached from the IVC-rack, CO$_2$ and other gaseous emissions start to build up. In IVCs without ventilation, the concentration of CO$_2$ increases by 2-8 % within two hours depending on the type of the cage, when mouse body weight per cage volume in each cage was 20 g/l (Krohn & Hansen. 2002). The exposure of 3 or 5 % CO$_2$ has been shown to lower systolic blood pressure and heart rate of SD male rats (Krohn et al. 2003c).

The move from traditional open cages to IVCs causes changes in physical environment inside the cage. It can be expected that at least temperature, RH, acoustic environment and light intensity are altered compared to open cages in this transition, especially if both systems are in the same space and room air is circulated through the IVC-system. Obviously the climatic conditions in the cage depend on those of the surrounding room as well as the air supply source and exhaust of the cage-rack (Scheer et al. not dated). Clough et al. (1995) noted that the physical environment in IVCs compared to open cages, displayed higher temperatures than in the room, higher RH, lower light intensity and elevated background sound level when the air fan was in the room.

The ambient temperature has a variety of effects on core temperature, weight gain, delivery rate, litter size, food and water intake, organ weights, haematological values, blood pressure, heart rate (HR), activity, O$_2$ consumption and CO$_2$ elimination in mice and rats (Pool & Stephenson 1977, Yamauchi et al. 1981, Gwosdow & Besch 1985, Swoap et al. 2004). The elevation in ambient temperature decreases activity in male Wistar rats (Pool & Stephenson 1977) and mean arterial pressure (MAP) and HR in female SD rats and NIH Swiss mice (Swoap et al. 2004). At high temperatures, inactivity is the animals’ first attempt to reduce heat production and thus to counteract the rising body temperature.

An ambient temperature above 30 °C decreases delivery rate, litter size and weaning rate in Wistar rats (Yamauchi et al. 1981). Although higher ambient temperatures in the IVCs have been reported (Clough et al. 1995), the elevation of 1-4 °C is not necessarily detrimental to reproduction; as shown by Tsai et al. (2003) who examined the long-term reproduction performance between DBA/2 mice housed in open cages and IVCs in the same room. However, in the IVCs the coefficients of variation were higher for most of the measured parameters (e.g. total number of litters or pups/dam and breeding index). This suggests that individual mice need more time to adapt to the IVCs than to the open cages.
Environmental noise has been shown to exert effects on cardiovascular function, hormones, reproduction, sleep and body and organ weights in laboratory rodents (Rabat 2007, Turner et al. 2007). Chronic intermittent noise elevates plasma corticosterone and rats do not seem to habituate to the noise (Strausbaugh et al. 2003). Animals have different hearing ability than humans; rodents e.g. are able to hear ultrasounds (> 20 kHz) non-audible to humans (Heffner & Heffner 2007). It has been postulated that IVC system may be associated with these ultrasounds, but Krohn et al. (2003b) were unable to detect ultrasounds originating from the ventilation of the IVC system. Rats have a different hearing sensitivity than humans and therefore the R-weighting (Björk et al. 2000) should be used in studies on the acoustic environment. Rats seem to adapt to repeated sound stimuli (Voipio 1997), but cage material, working style and hearing sensitivity all may change the sound pressure level in the rodent cage (Voipio et al. 2006). This study also detected higher sound exposure levels caused by stainless steel than polycarbonate cages, but calm and hurried working style made no difference with either of the materials. When the results were adjusted for rat and human hearing capabilities, differences were found in procedures conducted with both cage materials and both working styles. As a common trend, H-weighted (tailored for human audiogram) sound exposure levels were about 10 dB higher than those with R-weighting.

The cages in the IVC system have extra lids and this often leads to dimmer light inside the cages, although the illumination of the IVCs has been measured in only a few studies (Clough et al. 1995). Dark cages are better for albino rodents because prolonged periods of bright lighting have been shown to cause retinal damage in these animals (Gorn & Kuwabara 1967, Stotzer et al. 1970, Weisse et al. 1974). The intensity of illumination is crucial to retinal damage in rats; 8000 lx evokes photoreceptor damage in a couple of hours, whereas 194 lx does the same over a longer time period (Kuwabara & Gorn 1968, O’Steen et al. 1972). Furthermore, albino and pigmented rats are different in terms of visual acuity; pigmented Dark Agouti (inbred) and Long-Evans (outbred), and wild rats have grating thresholds around 1.0 cycle/degree (c/d), whereas in the albino rats Fisher344 (inbred), Sprague-Dawley (SD, outbred) and Wistar (outbred) the corresponding value is 0.5 c/d. Interestingly, the highest visual acuity has been found in F1-hybrid of F344 x BN with grating threshold of 1.5 c/d (Prusky et al. 2002). The study of Birch & Jacobs (1979) found the same results in acuity in albino and hooded pigmented rats, but in the hooded rats, the luminance level had no effect on spatial acuity.

1.4 Stress and stress indicators in laboratory rats

Stress is the outcome of external or internal factors – stressors – which can alter biological equilibrium (Pekow 2005). Stress induces changes in animals’ physiology, behaviour and biochemistry (Moberg & Mench 2000). Behavioural changes consist of grooming, appetite, activity, aggression, facial expression, vocalization, appearance, posture and response to handling; physiological changes e.g. temperature, HR and blood pressure, respiration, weight loss, blood cell
count and cell structure; and biochemical changes *e.g.* levels of corticosteroids, catecholamines, thyroxin, prolactin, beta-endorphin, ACTH, glucagon, insulin and vasopressin (Pekow 2005). There are many ways with which stress can be assessed *e.g.* animals’ behaviour, cardiovascular parameters and activity, and biochemical assays such as corticosterone determined from blood or faecal samples.

Stress may affect animal welfare if consequent adaptation has biological costs (Pekow 2005). Stress can be defined in three ways, according to its impact on animal wellbeing; neutral stress, eustress or distress. Neutral stress induces adaptive effects that are not harmful or beneficial for animals, eustress initiates a response that enhances animal wellbeing, and distress induces a harmful adaptive response.

During acute stress, the autonomic nervous system (ANS) is activated and hormones are released from the brain, peripheral nervous system and other organs. The sympathetic nervous system (SNS) evokes release of the catecholamines, adrenaline and noradrenaline, from adrenal medulla. The catecholamines increase HR and blood flow, and release glucagon from pancreas which improves glucose availability in blood.

In the brain, hypothalamic pituitary adrenal (HPA) –axis response to stressors is manifested by secretion of corticotrophin releasing factor (CRF). CRF triggers anterior pituitary to release adrenocorticotropic hormone (ACTH), which then stimulates release of glucocorticoids (corticosterone in rats and mice) from adrenal cortex (Matteri *et al.* 2000, Pekow 2005). In rats, CRF has been shown to play an important role during mild stress situations associated with increases in blood pressure, HR, body temperature ($T_b$) and locomotor activity (LA), but CRF does not contribute to cardiovascular and body temperature regulation in normal non-stress situations (Morimoto *et al.* 1993). The adrenals can also be activated during beneficial activities like mating, but in general it indicates that the animal is experiencing some difficulties in trying to cope, and levels of the adrenal products or the activities of adrenal enzymes, which are involved in the synthesis of catecholamine (*e.g.* adrenaline, noradrenaline and dopamine), are useful welfare indicators (Broom 1991).

HPA-axis activation to stressors is restrained by negative feedback exerted by glucocorticoids; an interaction with two types of receptors in the brain: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). Since corticosterone binds to MRs with much greater affinity than to GRs, MRs are mainly occupied in rats during periods of inactivity and in the morning hours, whereas GRs are occupied mainly during the dark period and after exposure to stress (Armario 2006). Furthermore, van den Buuse *et al.* (2002) showed MRs and GRs mediate differential and opposing actions on corticosterone in regulating sympathetic cardiovascular stress responses in rats.

Stress can be also chronic, seen in three different situations (Armario 2006):

a) continuous exposure to stressors for at least a few days or weeks

b) repeated exposure, *e.g.* on daily basis, to the same stressor for one or several weeks
c) chronic exposure to combination of different stressors which change in some way from day to day.
1.4.1 Cardiovascular parameters

In mammals, the SNS plays an important role in the maintenance of cardiac output to meet the demands placed on the organism by increasing both HR and cardiac contractility. The baroreceptor reflex regulates blood pressure and HR under normal conditions (d’Uscio et al. 2000). In freely moving animals, the cardiac neural drive responsible for a substantial fraction of spontaneous HR variability depends on both vagal and sympathetic activity, while blood pressure variability reflects only the vagal influence (Ferrari et al. 1987).

In laboratory rats, blood pressure and HR increase in stressful situations, e.g. when they are subjected to even common procedures (Sharp et al. 2002a, 2002b, 2003a, Azar et al. 2005). Van den Buuse et al. (2001) showed that novelty stress in rats, e.g. when they are exposed to an open field situation, caused an increase in blood pressure and HR, and concluded that these responses were attributable to increased SNS activity.

Restricted feeding has been shown to lower blood pressure (Young 1978, Einhorn et al. 1982, van Ness et al. 1997) and HR (Herlihy et al. 1992) in normotensive and spontaneously hypertensive rats (SHR). It seems that the food restriction lowers the activity of the SNS thus decreasing blood pressure (Young 1978, Einhorn et al. 1982, van Ness et al. 1997). Furthermore, restricted feeding appears to lower HR in old F344 rats, which has been suggested to result from enhanced baroreflex responsiveness (Herlihy et al. 1992).

1.4.2 Corticosterone and IgA

In rats, activation of the HPA axis increases the serum corticosterone level in response to stressful stimulation; this response is not, however, as rapid as that of ANS. Corticosterone release has been detected in serum as early as three minutes after ACTH injection (Siswanto et al. 2008), but if exposure to stressors continues for 15 min or more, the maximum serum corticosterone level is detected after 30-60 minutes (Armario 2006). Corticosteroid metabolites are excreted both into urine and faeces, but compared to serum corticosterone, they have not been detected in the samples until 6-10 hours later in urine and 4-12 hours later in faeces after stressful event (Bamberg et al. 2001, Royo et al. 2004, Lepschy et al. 2006, Siswanto et al. 2008, Abelson et al. 2009).

The level of serum corticosterone has been shown to increase in rats in many stressful situations, such as after cage cleaning and obtaining a vaginal smear (Honma et al. 1984), blood sampling (Sabatino et al. 1991, Haemisch et al. 1999), during immobilization (Sternberg et al. 1992, Dhabhar et al. 1995, Sarrieau et al. 1998, Schrijver et al. 2002, Márquez et al. 2004, Tamashiro et al. 2004), acoustic startle exposure (Glowa et al. 1992) and forced swimming test (Sternberg et al. 1992, Armario et al. 1995) and a couple of hours before a meal when rats are fed restrictly (Honma et al. 1984, Sabatino et al. 1991, Duclos et al. 2005). Circulating corticosterone levels have been shown to increase equally in dominant and subordinate male rats in response to 1 hour immobilization (Tamashiro et al. 2004). Plasma corticosterone secretion in rats seems to decrease with repeated immobilization and
blood sampling, \textit{i.e.} evidence of some adaptation to these procedures (Haemisch \textit{et al.} 1999, Márquez \textit{et al.} 2004).

In rats, the corticosterone excretion has been estimated to occur 16-80 \% via faeces and 25-80 \% via urine, and there are differences in excretion during the time of the day and between males and females (Bamberg \textit{et al.} 2001, Erikson \textit{et al.} 2004, Lepschy \textit{et al.} 2007, Abelson \textit{et al.} 2009). Assessment of stress sensitive molecules from faecal samples has a number of advantages: \textit{e.g.} animals are most often not disturbed by sampling. Laboratory rodents defecate several times a day (Cavigelli \textit{et al.} 2005) which enables monitoring of an individual animal for several consecutive days or months. Furthermore, there is no need to handle animals, there is a delay before corticosteroids appear in the faecal pellets; all this ensures that the corticosteroid levels in the samples are not affected by the sampling procedure (Bamberg \textit{et al.} 2001, Möstl & Palme 2002, Cavigelli \textit{et al.} 2005).

Prolonged stress may also lead to immunosuppression; \textit{e.g.} the levels of secretory immunoglobulin A (IgA) in saliva have been used to assess welfare status in different housing conditions (Guhad & Hau 1996). Another possibility is to quantify the IgA from faecal samples (Eriksson \textit{et al.} 2004, Pihl & Hau 2003, Royo \textit{et al.} 2004). Royo \textit{et al.} (2004) stated that stress-induced changes in IgA concentrations occur more slowly than changes in corticosteroids and consequently faecal IgA may be more useful for assessing long-term well-being, while faecal corticosterone is better at monitoring acute stress events.

The study of Eriksson \textit{et al.} (2004) showed that the excretion of the corticosterone and IgA into faeces and urine did vary between day and night but was rather similar during the daytime. In the dark phase, the amounts excreted in the urine increased dramatically whereas faecal corticosterone excretion exhibited only a moderate increase. The same has been shown with faecal IgA (Royo \textit{et al.} 2004), but some studies have shown higher faecal corticosterone secretion in the morning samples compared to the evening samples (Bamberg \textit{et al.} 2001, Pihl & Hau 2003, Royo \textit{et al.} 2004, Cavigelli \textit{et al.} 2005, Lepschy \textit{et al.} 2007). Furthermore, in female rats, the corticosterone levels vary with the estrus cycle; daily faecal corticoid levels were lowest on the day of estrus and rose progressively during metestrus, diestrus and proestrus (Cavigelli \textit{et al.} 2005). Furthermore, the basal serum corticosterone levels have been reported to be higher in the evening compared to the morning samples (Dhabhar \textit{et al.} 1993).

Corticosteroid measurements have been used in a few studies in rats to assess the value of furniture items, but the results have been often conflicting. The study of Belz \textit{et al.} (2003) showed that single-housed male and female SD rats with environmental enrichment had lower baseline plasma corticosterone levels than rats in standard cages, whereas another study with male Wistar rats detected significantly higher corticosterone levels in the animals housed in enriched cages (Moncek \textit{et al.} 2004). However, in the latter study, multiple combinations of various items were used and the effect of each single item could not be differentiated. Nevertheless, the combination did not seem to improve the housing
environment of the rats in terms of lowering their corticosterone levels.

The use of cortisol or corticosterone as an indicator of animal welfare in different housing methods has been criticized since cortisol and corticosterone levels are not always closely related to the mental or emotional states of animals and since the measures of a single hormone ignore the complex physiological reactions of animals to their environments (Rushen & de Passillé 1992). Furthermore, many studies in farm animals have also reported conflicting results; adrenal responsiveness after chronic stress has increased, decreased or there has been no change (Rushen 1991).

1.5 Telemetry as a study method in laboratory animal welfare

The traditional methods to measure blood pressure in the conscious animals are likely to cause an increase in the measurable blood pressure (Irvine et al. 1997). However, Abernathy et al. (1995) detected no difference between the tail cuff method and an implanted transmitter in the values of blood pressure and HR. The radiotelemetry transmitter allows measurement of the cardiovascular parameters in freely moving animals and hence values obtained are devoid of concomitant handling or restraint. For this reason, the results obtained with telemetry are more accurate. In addition to blood pressure and HR, telemetry allows measurement of many other physiological parameters such as ECG, 

Telemetry has become a widely used method in laboratory animal welfare science since it has many advantages over the more conventional techniques. Kramer et al. (2001) listed four advantages of radiotelemetry:
1. reduction of distress of conscious, freely moving laboratory animals
2. elimination of stress related to the use of restrainers
3. reduction of animals used
4. around-the-clock data collection

On the other hand, there are also potential harms which need to be considered when using telemetry: surgical implantation, physical impact of the device on the animal, and distress if animals are housed individually (Morton et al. 2003). In particular, the use of appropriate methods of anaesthesia and postoperative care with a proper analgesia are important, hence surgery procedural details should be described in detail in the scientific papers conducted with telemetry (Morton et al. 2003).

Modern transponders are totally implantable and animals can be group housed, but at present, only one animal can carry a transponder in the cage, because in most of the available devices the signal is transmitted at only one frequency. However, the devices with several frequencies are now entering the market. The report of the Hawkins et al. (2004) recommends keeping animals group housed in telemetry studies unless there are clear contraindications to this choice.

Cardiovascular parameters e.g. HR and blood pressure increase after handling and restraint, (Irvine et al. 1997, Kramer et al. 2000, Batūraitė et al. 2005) indicating that those parameters can be considered as stress indicators. Indeed telemetry has been used to assess an animal's response to the handling or experimental procedures, e.g. a rat’s response to the IG-gavage or cage changing procedures, but also to different kinds of housing, such as
flooring, enrichment and timed and restricted feeding. The articles examined telemetry in studies related to animal welfare in rats are listed in Table 1.2.

In undisturbed conditions, blood pressure, HR and LA of laboratory rats follow a circadian rhythm; all exhibiting lower values in the light phase (Saleh & Winget 1977, Witte et al. 1998, van den Buuse 1994, 1999, van den Brant 1999). The diurnal rhythm is controlled by the circadian oscillator, which is located in the suprachiasmatic nuclei in the hypothalamus. Induced lesions to the circadian oscillator have been shown to interfere with many physiological activities, e.g. blood pressure, HR and LA (Saleh & Winget 1977, Janssen et al. 1994, Sano et al. 1995), wake rhythm and sleep (Ibuka & Kawamura 1975, Sei et al. 1995, Sei et al. 1997) and timed feeding (van den Buuse 1999). Indeed, most of the studies on the circadian rhythm as related to cardiovascular parameters and LA have been done with telemetry.

Telemetry has also been used to assess blood pressure, HR, LA and body temperature of various rat strains (van den Buuse 1994, van den Brant 1999), effect of ambient temperature (Swoap et al. 2004) and to compare adult and old rats (Zhang & Sannajust 2000). Van den Brant (1999) showed that when the SBP of the wild “ancestor” rat is compared to inbred rats, the strains can be divided into two categories: “hypotensive“ and “hypertensive”. Differences between strains have also been detected in HR and LA (van den Buuse 1994, van den Brant 1999).

### 1.6 Restricted feeding in rats

The overwhelming majority of laboratory rodents are fed *ad libitum*, i.e. food is available all the time. However, there is evidence that *ad libitum* feeding causes obesity and increases the incidence of neoplasia, kidney, heart and other organ diseases in rats (Yu et al. 1982, Roe 1994, Roe et al. 1995, Lipman et al. 1999, Hubert et al. 2000). Rats on restricted feeding live longer; their survival curve shifts to the right compared to *ad libitum* fed rats, with the difference being about one year (Yu et al. 1982, Yu et al. 1985, Hubert et al. 2000). However, different physiological and behavioural consequences are associated with food restriction (Toth & Gardiner 2000).

Keenan et al. (1999) argued that *ad libitum* feeding is the worst standardized factor in rodent bioassays. In long-term studies, the longevity of the animals is compromised due to neoplasia and degenerative diseases, and this interferes with the sensitivity of the study and necessitates more animals being used. Masoro (2005) has reviewed the caloric restriction (CR) studies on aging and suggested that the obesity and CR are a consequence of a combination of different mechanisms. However, it has been proven that the use of restricted feeding in carcinogenicity tests can reduce the incidence of tumours in the control animals, which lowers the number of animals needed to obtain significant results (Beynen 1992).

Laboratory rats should be housed in groups (Council of Europe 2006, EU 2007b), which is also the preferred method. However, when animals are group housed, there is no practical or effective way to restrict the food intake of all individuals within the group at the same
Table 1.2 Summary of telemetry studies on refinement and reduction in rats. Abbreviations: MAP = mean arterial pressure, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure, HR = heart rate, LA = locomotor activity, $T_b$ = body temperature.

<table>
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time. In the group-housing situation, the dominant animal eats more than the others (Beynen 1992, Ritskes-Hoitinga & Chwalibog 2003). In solitary animals arranging restricted feeding is technically straightforward, but it may change the diurnal rhythm of the animals.

There are two approaches used in food or caloric restriction studies in rats (Claassen 1994). In meal feeding rats have access to food for only a few hours during the day (Saito et al. 1975, Stephan 1984, Strubbe, & Alingh Prins 1986, Roe et al. 1995, van den Buuse 1999). In another approach, a certain amount of food as a single portion is offered daily (Vermeulen et al. 1997, Markowska 1999, Hubert et al. 2000). One common feature of these methods is that rats have to be housed on their own. Furthermore, with the single-meal feeding, plasma corticosterone levels have been shown to increase for a couple of hours before the meal and decrease soon thereafter (Honma et al. 1984, Sabatino et al. 1991). Thus, these kinds of caloric restriction methods seem to be stressful to rats.

Restricted feeding has been shown to decrease blood pressure (Young 1978, Einhorn et al. 1982, van Ness et al. 1997) and HR (Herlihy et al. 1992) in rats. It was concluded that food restriction had reduced the activity of the SNS causing a hypotensive effect (Young 1978, Einhorn et al. 1982, van Ness et al. 1997), enhanced baroreflex responsiveness as well as decreasing HR in old F344 rats (Herlihy et al. 1992).

Nocturnal animals, such as rats, forage and eat mainly during the dark phase. In laboratory animal facilities, rats eat most of their daily food (70-95 %) during the dark phase if the food is available ad libitum (Zucker 1971, Spiteri 1982, Strubbe et al. 1986b, Strubbe & Alingh Prins 1986); in fact eating during the dark seems to be genetically determined in rats (Ritskes-Hoitinga & Strubbe 2004). Normal feeding activity of rats consists of two peaks during the dark, the first one at the beginning of the dark phase and the other at the end (Spiteri 1982, Strubbe et al. 1986a), and it has been shown that when ad libitum feeding was reinstated after a restricted feeding schedule, the rats will instantly revert to their original feeding pattern (Spiteri 1982, Strubbe et al. 1986b). When rats are fed once a day or the food deprivation period has been longer than six hours, locomotion behaviour (Yu et al. 1985, Vermeulen et al. 1997) and running wheel activity (Duclos et al. 2005) increase; both likely as consequences of food searching behaviour.

Normal feeding activity of rats follows the circadian rhythm (Stephan 1984, Strubbe et al. 1987, Sano et al. 1995, Ritskes-Hoitinga & Chwalibog 2003). When rats are fed with a single meal or they have certain amount of food once a day, this normally happens during the light phase because of the facility's working hours. In this situation, animals consume all of the offered food right away, which interferes with species-specific eating patterns and compromises digestive physiology. In rats, this may lead to major phase changes in biochemical and physiological functions of the digestive system in dark active animals. For example, changes have been observed in the levels of serum insulin and glucose (Strubbe & Alingh Prins 1986, Strubbe et al. 1987, Rubin et al. 1988), mucosal enzymes of small intestine (Saito et al. 1975) and bile flow (Ho &
Drummond 1975). Last, but not least, the altered eating times have been shown to exert an impact on blood pressure, heart rate and behavioural activity in rats (van den Buuse 1999, Curtis et al. 2003).

One way to combine group housing and restricted feeding is to provide foraging items. The review of Johnson & Patterson-Kane (2003) lists the theoretical backgrounds of foraging items for rats. They state four reasons for providing foraging items for laboratory rats:

1. foraging ethology
2. optimal foraging theory
3. contrafreeloading
4. foraging items used in other species

The ethological argument originates from the fact that in their natural environment, rats need to search, identify, procure and handle material in order to acquire food. According to the optimal foraging theory, in rats there are other motives in addition to hunger to encourage foraging, e.g. net energy intake (Johnson & Patterson-Kane 2003).

Contrafreeloading is a phenomenon where animals would rather work for food than eat from a freely available food source. Carder & Berkowitz (1970) and Neuringer (1969) reported that even if the rats had free access to food, they would rather earn their food as long as the work demand was low. Coburn & Tarte (1976) found that rats living in an impoverished environment pressed a lever more often than rats living in an enriched environment, and suggested this to result from the increased possibility for activity. The review of Inglis et al. (1997) notes that contrafreeloading can occur in many different species, not only in rats. Inglis et al. (1997) also list the factors that have an effect on the level of contrafreeloading: prior training, level of food deprivation, required effort, stimulus change, environmental uncertainty, rearing conditions, manipulation of the environment and the nature of the foraging task.

Foraging items have been widely used in other species and previous studies have demonstrated the success of foraging items in improving welfare (Johnson & Patterson-Kane 2003). Non-device foraging items provide variety in the food and the location of the food in the enclosure, e.g. frozen food, changes in food size, scatter feeding, hiding the food and live food. With foraging devices, the animal must manipulate the item in order to access the food, i.e. food balls and puzzle feeders (Johnson & Patterson-Kane 2003).

There are also studies intended to develop foraging items for laboratory rats. In the study of Johnson et al. (2004) rats had access to their diet only via a one cm wide slot or alternatively they had a “foraging device”, where the pellets were under gravel. With the slot feeding, rats ate longer but consume less food surprisingly with no effect on weight. The rats preferred eating from the “foraging device”, gained more weight than ad libitum fed controls eventhough work was required to access to food.

Cover & Barron (1998) introduced a diet optimization feeder including seven carousel wells for every weekday and a semi-automated filling station. This feeder provided only a certain amount of daily food for rats, which can evoke meal-feeding problems as discussed above. A fourth method studied incorporation of largely indigestible sugar beet pulp fibre into the chow; weight gain reduction was achieved, but the method also resulted in an enlarged gastrointestinal tract -
especially caecum (Eller et al. 2004).

1.7 Cage furniture in laboratory rats

In laboratory rats, various items made of diverse materials have been used to furnish animal cages, as can be seen in Table 1.3. In most of the studies, the cage items have been called “enrichment”, which is defined in many ways depending on the perspective of the definer. Newberry (1995) defined environmental enrichment as “an improvement in the biological functioning of captive animals resulting from modifications to their environment”. Evidence of improved biological functioning was defined e.g. increased lifetime for reproduction, increased inclusive fitness, or improved health. Chamove (1989) used behaviour to define enrichment; the aim of enrichments is to increase “desirable” behaviour (e.g. foraging) and reduce “undesirable” behaviour (e.g. stereotyped behaviour or hair-pulling); i.e. enrichment allows the animals to exhibit species specific behaviours. Purves (1997) stated the enrichment improves the life of laboratory rodents, and creates less variability in experimental outcomes, and thus reduces the number of animal needed.

Newberry (1995) listed problems in the enrichment studies; the control environment in different studies varies from wire bottom cages with one animal to large pens with several animals, and added objects from single item to diverse combination of items. Furthermore, the term “enrichment” means an improvement, but according to Newberry (1995), the term is applied to different types of environmental change (e.g. social, physical, sensory) rather than the outcome of studies.

Environmental enrichment has been shown to have significant effects on growth and behaviour of male rats (Zaias et al. 2008), and enhance habituation of exploratory activity in response to novelty and improved spatial learning and memory (Schrijver et al. 2002). The early study of Cummins et al. (1977) showed differences in brain development in Wistar rats that were housed with sensory enrichment or a deprived environment. Based on these results they proposed a developmental model for environmental enrichment. One essential feature of the model is that there exists an element of neural development associated with cells that fully mature only in response to sensory stimulation. The neural development is represented on a percentage scale (y-axis), where 100 % means that the development cannot proceed anymore, and the sensory stimulation is from minimal to optimal scale (x-axis).

Rats prefer a cage with a shelter (Townsend 1997, Manser et al. 1998b, Patterson-Kane et al. 2001, Patterson-Kane et al. 2003). The reason for this preference may be that shelters provide protection from light for rats (Manser et al. 1998a, Eskola et al. 1999). Rats with a furnished environment are more active than those without a shelter (van der Harst et al. 2003) and its presence in the cage decreases fearfulness (Townsend 1997).

The European regulations on laboratory rodents mandate the provision of sufficient nest material for nest building; if that is not possible, a nest box should be provided (Council of Europe 2006, EU 2007b). Since rats are poor nest-builders (Jegstrup et al. 2005), they must be provided with cage furniture for this purpose. Furniture with a cover and dividing walls in the cage area may
### General introduction

**Table 1.3** Summary of studies on cage items used in rats.

<table>
<thead>
<tr>
<th>Study</th>
<th>Strain/stock</th>
<th>Cage items</th>
<th>Topic/Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belz et al. 2003</td>
<td>SD</td>
<td>Kong Toy®, Nestlet®</td>
<td>Plasma ACTH, corticosterone</td>
</tr>
<tr>
<td>Bradshaw &amp; Poling 1991</td>
<td>SD</td>
<td>Paper towels, wood chips, plastic pipe, wood platform</td>
<td>Direct observations with the items</td>
</tr>
<tr>
<td>Burke et al. 2007</td>
<td>SD</td>
<td>Plastic hiding hut, polyvinyl chloride tube, plastic climbing platform, one toy, four wooden chews</td>
<td>Open-field activity, electrophysiological and morphometric outcome measures with moderate thoracic spinal core injury</td>
</tr>
<tr>
<td>Chmiel &amp; Noonan 1996</td>
<td>Long-Evans</td>
<td>15 items e.g. different kinds of balls</td>
<td>Direct observations with the items</td>
</tr>
<tr>
<td>Eskola et al. 1999</td>
<td>Wistar</td>
<td>A block with drilled holes, rectangular tube</td>
<td>Behaviour, gnawing volume, growth of rats</td>
</tr>
<tr>
<td>Holm &amp; Ladewig 2007</td>
<td>SD</td>
<td>Aspen tube, aspen gnawing sticks and wood-wool nesting material</td>
<td>Level pressing activity in rats in object enriched or non-enriched environment</td>
</tr>
<tr>
<td>Johnson et al. 2004</td>
<td>Wistar</td>
<td>Modified food hopper, gnawing sticks, “foraging device”</td>
<td>Behaviour, food consumption, weight gain, choices in preference test</td>
</tr>
<tr>
<td>Lawson et al. 2000</td>
<td>SHR</td>
<td>PVC drainpipe, golf balls, running wheel</td>
<td>SBP, DBP, HR, activity</td>
</tr>
<tr>
<td>Manser et al. 1998a</td>
<td>SD</td>
<td>Nest box, nesting material</td>
<td>Time spent with objects in preference tests</td>
</tr>
<tr>
<td>Manser et al. 1998b</td>
<td>SD</td>
<td>Nest box, nesting material</td>
<td>The weight rats lifted to reach the cage with studied objects</td>
</tr>
<tr>
<td>Moncek et al. 2004</td>
<td>Wistar</td>
<td>Toys, tunnel, swing, running wheel</td>
<td>Adrenal weight, ACTH, corticosterone, Behavioural activities</td>
</tr>
<tr>
<td>Orok-Edem &amp; Key 1994</td>
<td>Lewis</td>
<td>Tongue depressor, hanging wooden block</td>
<td></td>
</tr>
<tr>
<td>Patterson-Kane et al. 2001</td>
<td>Hooded Norway</td>
<td>40 different toys and pieces of equipment e.g. large marble, wooden block, chicken wire ball, shredded paper, tin nest box</td>
<td>Choices in preference tests</td>
</tr>
<tr>
<td>Patterson-Kane 2003</td>
<td>Wistar</td>
<td>Seven different kind of shelters</td>
<td>Choices in preference tests</td>
</tr>
<tr>
<td>Study</td>
<td>Strain/stock</td>
<td>Cage items</td>
<td>Topic/Parameters</td>
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</tr>
<tr>
<td>Schrijver <em>et al.</em> 2002</td>
<td>Lister Hooded</td>
<td>A thick layer of bedding material, shelves, wooden branches, hay, a rope, plastic tunnels, a hut</td>
<td>Results of behavioural tests (open field, L/D box, Morris water maze), plasma ACTH and corticosterone</td>
</tr>
<tr>
<td>Sharp <em>et al.</em> 2005</td>
<td>SD, SHR</td>
<td>Simulated burrow, gnawing and food foraging items, shredding and nesting items.</td>
<td>SBP, HR, Activity</td>
</tr>
<tr>
<td>Townsend 1997</td>
<td>Wistar</td>
<td>Shelter made of mouse cage</td>
<td>Behaviour</td>
</tr>
<tr>
<td>Van den Harst <em>et al.</em> 2003</td>
<td>Wistar</td>
<td>Shelter, tunnel-shaped compartment, gnawing sticks</td>
<td>Behaviour</td>
</tr>
<tr>
<td>Williams <em>et al.</em> 2008</td>
<td>Lister-Hooded</td>
<td>A shelter, a box shape, a post and a car – all made of Lego®</td>
<td>The frequency of first introduction and time spent with objects in preference tests</td>
</tr>
<tr>
<td>Zaiaš <em>et al.</em> 2008</td>
<td>SD</td>
<td>Plastic tunnels and balls, paper nestlets</td>
<td>Body weight, food consumption, activity</td>
</tr>
</tbody>
</table>

be useful to allow the rats to collaborate or avoid contact with other group members (Stauffacher 2002). A cage divider can be used for separating individual animals but also enabling social interaction at the same time (Boggiano *et al.* 2008). The regulations are the same for all laboratory rodents, *i.e.* rats, mice, gerbils, hamsters and guinea pigs. Nonetheless, all rodent species, and strains and stocks within a species display differences (see Table 1.5) and specific needs, which require detailed consideration and implementation. Nonetheless, the study of Sharp *et al.* 2005 has stated that it is difficult to make generalized recommendations for the animal care community with regard to rat enrichment programs.

The cage item has been made of different materials, such as plastic, wood and cardboard. The items made of wood are beneficial because then they are usually the same material as the bedding. Cage items that are made of organic material have been shown to emit volatile compounds, like pinenes, but these emissions can be reduced by autoclaving prior to use (Nevalainen & Vartiainen 1996). Bedding made of soft wood (spruce, cedar, pine) has been reported to increase hexobarbital oxidase activity and shorten sleep time in mice (Vesell 1968). The presence of volatile compounds in bedding can induce hepatic microsomal enzymes and hence modify pharmacological effects, *e.g.* duration of sleeping time (Ferguson, 1966, Vesell, 1968, Wade *et al.*, 1968; Sabine, 1975, Cunliffe-Beamer *et al.*, 1981, Nielsen *et al.*, 1984, Weichbrod *et al.*, 1988), but also these compounds can influence some aspects of...
endocytosis (Buddaraju & Van Dyke, 2003). Aspen tubes as a cage items have been shown to endure several bouts of sanitation without increasing microbiological burden (Voipio et al. 2008). Old cast-off bottles have also been used as cage items for laboratory rodents and are considered to have no effect on the animals. However, bottles are normally made of polycarbonate, which has been reported to leach bisphenol A towards end of their useful life (Howdeshell et al. 2003), which is a compound with estrogenic activity (Krishnan et al. 1993) and thus old polycarbonate bottles are not recommended to be used as cage items.

1.8 Cage change and IG-gavage
Laboratory rats may be subjected to a variety of experimental procedures, e.g. IG (intragastric)-gavage, which may have a negative effect on animal welfare. Similar untoward effects can also result from routine animal care procedures, such as weekly cage cleaning. This chapter focuses on these two common procedures in animal facilities, and summarizes studies published so far (Table 1.4).

In animal facilities, rats are changed to a clean cage either once or twice per week depending on cage density, bedding material and cage type. This routine procedure has been shown temporarily to increase blood pressure, HR, activity (Schnecko et al. 1998, Duke et al. 2001, Sharp et al. 2002a, Sharp et al. 2002b, Sharp et al. 2003a, Sharp et al. 2003c, Azar et al. 2005) and behaviour (Saibaba et al. 1996, Burn et al. 2006a, Burn et al. 2006b). Moreover, it has been shown that cage cleaning frequency (Burn et al. 2006a, Burn et al. 2006b), the time of the cleaning (Schnecko et al. 1998), the type of the bedding material (Burn et al. 2006a), the light intensity and the length of the dark period (Azar et al. 2008) all alter the response intensity. It is the transfer procedure itself that is reflected in blood pressure and HR after the cage change rather than the novelty of the environment.

In their natural environment, rats have dominance hierarchies and fighting is related to their territory, not for any specific object (Barnett 1958). The study of Burn et al. (2006a) used the term “skirmishing” to describe the pattern of behaviours, which were assumed to be aggressive in rats. The skirmishing frequency increased significantly within 15 min after a cage change, but returned to the normal level within next 15 min (Burn et al. 2006a). Since they are exploratory animals, rats start to investigate their new surroundings by ambulating and rearing (Hughes 1968) and indeed cage change seems to increase the LA in rats (Burn et al. 2006b, Saibaba et al. 1996, Schnecko et al. 1998). The behaviour patterns of rats change in clean cages; grooming, eating, drinking, resting, rearing and bedding manipulation all decrease, while walking and skirmishing frequencies increase immediately after the rats are placed in the clean cage (Burn et al. 2006b).

Rats are known to have a good sense of smell; the number of identified specific olfactory receptor genes on the cilia of the olfactory neurons is high - 1493; in dogs the corresponding number is 1094; therefore smell is the rats’ primary sense for monitoring their environment (Quignon et al. 2005). In the rat cage, furniture can serve as an odour cue making the new cage environment more...
Niina Kemppinen: 2Rs outcomes of cage furniture and restricted feeding in laboratory rats

Table 1.4 Summary of studies on cage change or IG-gavage in rats. Abbreviations: MAP = mean arterial pressure, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure, HR = heart rate, T<br> = body temperature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Strain/stock</th>
<th>Topic</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abou-Ismail et al. 2008</td>
<td>Wistar</td>
<td>Cage changing</td>
<td>Behaviour, organ weights</td>
</tr>
<tr>
<td>Alban et al. 2001</td>
<td>Wistar</td>
<td>IG-gavage volumes</td>
<td>Open-field arena behaviour, BT</td>
</tr>
<tr>
<td>Azar et al. 2005</td>
<td>SD</td>
<td>Cage change</td>
<td>MAP, HR</td>
</tr>
<tr>
<td>Bonnichsen et al. 2005</td>
<td>SD</td>
<td>Stress duration and gavage volume</td>
<td>MAP, HR, T&lt;br&gt;</td>
</tr>
<tr>
<td>Brown et al. 2000</td>
<td>SD</td>
<td>Different vehicles and dose volume</td>
<td>Plasma corticosterone, lung weight</td>
</tr>
<tr>
<td>Burn et al. 2006a</td>
<td>Wistar and SD</td>
<td>Cage changing frequency</td>
<td>Behaviour, scoring of chromodacryorrhoea, wounds, handleability</td>
</tr>
<tr>
<td>Burn et al. 2006b</td>
<td>Wistar and SD</td>
<td>Cage changing frequency</td>
<td>Behaviour, scoring of chromodacryorrhoea</td>
</tr>
<tr>
<td>Burn et al. 2008a</td>
<td>Wistar</td>
<td>Cage cleaning frequency</td>
<td>Reproduction, pup mortality</td>
</tr>
<tr>
<td>Burn et al. 2008b</td>
<td>Lister Hooded</td>
<td>Preference for clean or soiled cages</td>
<td>Behaviour, ammonia, food eaten, amount of faecal pellets</td>
</tr>
<tr>
<td>Duke et al. 2001</td>
<td>SD</td>
<td>Cage cleaning</td>
<td>MAP, HR</td>
</tr>
<tr>
<td>Honma et al. 1984</td>
<td>Wistar</td>
<td>Cage cleaning</td>
<td>Plasma corticosterone</td>
</tr>
<tr>
<td>Saibaba et al. 1996</td>
<td>SD</td>
<td>Cage cleaning time</td>
<td>Behaviour</td>
</tr>
<tr>
<td>Schnecko et al. 1998</td>
<td>SD</td>
<td>Cage cleaning time</td>
<td>SBP, DBP, HR, Activity</td>
</tr>
<tr>
<td>Sharp et al. 2002a</td>
<td>SD</td>
<td>Cage change</td>
<td>MAP, HR</td>
</tr>
<tr>
<td>Sharp et al. 2002b</td>
<td>SD</td>
<td>Cage change</td>
<td>MAP, HR</td>
</tr>
<tr>
<td>Sharp et al. 2003a</td>
<td>SD</td>
<td>Cage change</td>
<td>MAP, HR</td>
</tr>
<tr>
<td>Sharp et al. 2003c</td>
<td>SD</td>
<td>Cage change</td>
<td>HR</td>
</tr>
<tr>
<td>Ökva et al. 2006</td>
<td>Wistar</td>
<td>IG-gavage</td>
<td>SBP, DBP, HR</td>
</tr>
</tbody>
</table>

familiar if it is transferred to the clean cage with the animals; the presence of the old item in the new cage has been shown to reduce the aggressive behaviour triggered by regrouping (Burn et al. 2006b).

The HR of the rats appeared to increase even by moving the cage to a different place in the rack (Gärtner et al. 1980), and plasma corticosterone levels increased significantly 15 min after arrival of rats into a clean cage (Honma et al. 1984). Schnecko et al. (1998) have studied the impact of a cage changing...
time on HR and blood pressure and they reported that if the change took place in the morning - during the resting period - SBP, DBP and HR responses were larger than following the same procedure during the active period i.e. the evening. Moreover, Abou-Ismail et al. (2008) showed that if rat cages were changed in the light period, they slept less, had more chromodacryorrhea, displayed a smaller thymus weight, had higher levels of aggression and exhibited less furniture-directed behaviour. Consequently, the authors suggested that if husbandry procedures were done during the dark rather than the lights-on, this might improve rat welfare. However, this kind of timing is rather impractical.

The studies into IG-gavaging have demonstrated that the blood pressure and HR increase seen in rats immediately after the procedure and the response can be seen for 30-60 min beyond the procedure (Bonnichsen et al. 2005, Ökva et al. 2006). Moreover, the plasma corticosterone levels of rats are known to increase after an IG-gavage (Brown et al. 2000). The choice of the administration volume (Alban et al. 2001, Bonnichsen et al. 2005, Brown et al. 2000, Ökva et al. 2006) and a suitable probe material (Ökva et al. 2006) have both been shown to lead to refinement of the procedure.

The study of Ökva et al. (2006) showed essentially the same responses in blood pressure and HR to cage change and IG-gavage in Wistar rats. However, the corticosterone level seems to increase more when rats are moved to a novel environment than to short-term handling (Seggie & Brown 1991, Haemisch et al. 1999, Márquez et al. 2004), taking a vaginal smear (Honma et al. 1984) or when rats are placed into restrainers (Sternberg et al. 1992, Dhabhar et al. 1993, Dhabhar et al. 1995, Sarrieau et al. 1998, Márquez et al. 2004). Cloutier & Newberry (2008) have tried to use classical conditioning to evaluate two stressful procedures, handling and a saline injection, paired with a rewarding experience. However, the rewards, food treats or tickling, did not alleviate the stress response in SD rats.

The IG-gavage is a short-term procedure, usually considered more invasive than handling, and one common feature to both gavage and handling is that the rats are returned to the familiar home cage. In the cage change procedure, the animals are relocated into a new environment with new odours; hence it is no surprise that the response to cage change is more intense.

Only a few of studies have dealt with the effects of cage change and IG-gavage procedures with added cage items. Sharp et al. (2005) studied various potentially stressful procedures, using both SD and SHR rat strains, and showed that a multifaceted enrichment program over a week had no effect on HR and SBP responses when the rats were placed in a standard rodent restrainer for 60 min. However, when the rats were removed from the restrainer, a secondary increase in HR and SBP occurred, which was significantly lower in enriched rats compared to their non-enriched counterparts in both strains. Moreover, SD and SHR rats housed in an enriched environment displayed lower HR and SBP responses to many of the studied procedures, such as removal of a cage mate, tail vein injection and exposure to the odour.
of urine and faeces of stressed male or female rats.

1.9. Strain differences in rat

It is well known that different strains and stocks of laboratory rat are not identical or even very similar e.g. in their physiology, behaviour and biochemical characteristics. Examples of reported differences between strains and stocks can be seen in Table 1.5. At least some of these differences can be attributed to their genetic background. Indeed, some studies claim that these variables could be used for the quantitative trait loci (QTL) analysis of inbred strains (Lipman et al. 1999, Avsaroglu et al. 2007). QTL analysis is a statistical method for evaluating the alleles that occur in a locus and the phenotypes which they control.

Festing et al. (2002) suggested that by studying various defined genotypes with a factorial design for evaluating the strains, then better precision and applicability within the species could be achieved. Brown Norway (BN) and Fisher 344 (F344) strains differ in various aspects of their physiology, biochemical characteristics and behaviour (Table 1.6). These two strains are commonly used in aging studies (Spangler et al. 1994, Lipman et al. 1996, Lipman et al. 1999, Sheridan et al. 2007). Sheridan et al. (2007) stated the BN and F344 rats are useful models for investigation of the molecular mechanisms because they have the same kind of interindividual variation in collateral growth capacity and a similar impact of age on compensation as in clinical observations.

Both BN and F344 rats develop spontaneous lesions at older ages. Lipmann et al. (1999) detected 80 and 58 different types of lesions in BN and F344 rats, respectively. The frequency of lesions was highest in adrenal glands, kidneys, lungs and pancreas in BN rats and in eyes, heart, lungs and kidneys in F344 rats. In addition, the F344 rats had higher incidence for leukaemia.

Van den Brant et al. (1999) studied blood pressure, HR and activity in six inbred rat strains, and they categorised F344 as a “hypertensive” and the BN as a “hypotensive” rat strain compared to wild rats. The difference in day-night activity of BN rats was also lower compared to F344 and van den Brant and co-workers concluded that the BN strain no longer possessed the typical rodent nocturnal activity. However, in a study of nest building behaviour (Jegstrup et al. 2005) it was revealed that there was a closer genetic relationship between BN and wild rats than was the case with the two other strains studied (BDIX and LEW). On the other hand, F344 rats have been considered to be “stress hyper-responsive” because of their high corticosterone responses to restraint and in behavioural studies (Dhabhar et al. 1995).
Table 1.5 Summary of differences in laboratory rat strains and stocks. Abbreviations: BP = blood pressure, HR = heart rate, ACTH = adrenocorticotropic hormone, CBG = corticosteroid-binding globulin, CRF = corticotropin-releasing factor, GR = glucocorticoid receptor, MR = mineralocorticoid receptor, HPA = hypothalamic-pituitary-adrenal. Strains and stocks: BN = Brown Norway, DA = Dark Agouti, F344 = Fischer 344, LE = Long Evans, Lew = Lewis, SD = Sprague Dawley, SHR = Spontaneously Hypertensive Rat, WIST = Wistar, WKY = Wistar-Kyoto, WF = Wistar-Furth.

<table>
<thead>
<tr>
<th>Study</th>
<th>Strains/stocks</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armario et al. 1995</td>
<td>BN, F344, Lew, SHR, WKY</td>
<td>Behavioural and endocrine response to forced swimming stress</td>
</tr>
<tr>
<td>Avsaroglu et al. 2007</td>
<td>ACI, BN, COP, F344, Lew, SHR, WAG, WKY</td>
<td>Response to anaesthetics and analgesics</td>
</tr>
<tr>
<td>Bean et al. 2008</td>
<td>F344, LE</td>
<td>Housing density</td>
</tr>
<tr>
<td>Behmoaras et al. 2005</td>
<td>LE, F344, WF, WAG, BN, Lew, LOU</td>
<td>Aortic elastin and collagen</td>
</tr>
<tr>
<td>Dhabhar et al. 1993</td>
<td>F344, Lew, SD</td>
<td>Plasma CBG and corticosterone, and adrenal receptor activation response to stress</td>
</tr>
<tr>
<td>Dhabhar et al. 1995</td>
<td>F344, Lew, SD</td>
<td>Plasma ACTH, CBG and corticosterone, and adrenal receptor activation response to stress</td>
</tr>
<tr>
<td>Duclos et al. 2005</td>
<td>BN, F344, Lew</td>
<td>HPA-axis activity</td>
</tr>
<tr>
<td>Glowa et al. 1992</td>
<td>F344, Lew, SD</td>
<td>Serum corticosterone response to acoustic stimuli</td>
</tr>
<tr>
<td>Gómez et al. 1996</td>
<td>BN, F344, Lew, SHR, WKY</td>
<td>HPA response to chronic stress</td>
</tr>
<tr>
<td>Gómez et al. 1998</td>
<td>BN, F344, Lew, SHR, WKY</td>
<td>Glucocorticoid feedback on HPA-axis</td>
</tr>
<tr>
<td>Irvine et al. 1997</td>
<td>SHR, WKY</td>
<td>Influence of restraint on BP</td>
</tr>
<tr>
<td>Jegstrup et al. 2005</td>
<td>BN, BDIX, Lew</td>
<td>Nest-building behaviour</td>
</tr>
<tr>
<td>Lemaire &amp; Mormède 1995</td>
<td>WIST, LE, BHR</td>
<td>BP and HR during chronic social stress</td>
</tr>
<tr>
<td>Lipman et al. 1996</td>
<td>BN, F₁(F344 x BN), F₁(BN x F344)</td>
<td>Pathologic characterisation</td>
</tr>
<tr>
<td>Lipman et al. 1999</td>
<td>BN, F344, F₁(BN x F344)</td>
<td>Effect of genotype and diet on age-related lesions</td>
</tr>
<tr>
<td>Marissal-Arvy et al. 1999</td>
<td>BN, F344</td>
<td>Corticosteroid receptor efficiency and regulation</td>
</tr>
<tr>
<td>Marissal-Arvy &amp; Mormède 2004</td>
<td>BN, F344</td>
<td>Excretion of electrolytes</td>
</tr>
<tr>
<td>Nemelka et al. 2008</td>
<td>F344, LE</td>
<td>Housing density</td>
</tr>
<tr>
<td>Ohtsuka et al. 1997</td>
<td>BN, F344</td>
<td>Response to formaldehyde inhalation</td>
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<tr>
<td>Study</td>
<td>Strains/stocks</td>
<td>Topic</td>
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<td>F344, Lew, LE, SD, WIST</td>
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Table 1.6 Summary of verified differences between BN and F344 rats. Abbreviations: GR = glucocorticoid receptor, MR = mineralocorticoid receptor, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate.

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1.10 Aims of the present study

The general aim of this study was to identify refinement and reduction methods for rat housing and two commodity procedures.

The specific aims were:

1. To characterize and compare physical parameters in a common situation, where IVC-racks are kept in the same room with open cages (Chapter II).

2. To assess whether a novel system of food restriction, based on the work for food principle, would have any effect on weight gain over a short period, food utilisation and amount of wood gnawed in adult rats and whether their time budget would differ from ad libitum fed rats (Chapter III).

3. To determine whether isolating walls, cage tube or restricted feeding change responses to routine cage change or IG-gavage in both open and IVCs (Chapter IV).

4. To evaluate the impact of large cage furniture items on LA, cardiovascular parameters, and on faecal excretion of corticosteroid metabolites and IgA, to determine how applicable the results are, and whether there would be habituation to the items (Chapters V and VI).
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General introduction

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CHAPTER II

Exposure in the shoebox: Comparison of physical environment of IVCs and open rat cages.


Exposure in the Shoebox:  
Comparison of Physical Environment of IVCs and Open Rat Cages

by Niina Kemppinen*, Anna Meller†, Erkki Björk‡, Tarja Kohila‡ & Timo Nevalainen§

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Summary

New caging and innovative items for more structured environment within the cage have been introduced. Many of these innovations cannot be seen as 'pure' or individual procedures, but rather they represent a mixed exposure with a multitude of operant factors, some possibly having an impact on animals and research. One kind of new caging system is the individually ventilated cage (IVC), where each cage receives its own non-contaminated airflow, primarily designed for health status maintenance and occupational safety. Even though those cages may be the same as those used in open cage systems, the physical environment inside the cage may not identical. Comparison between cage types is difficult without characterization of the physical environment, because the change may involve alterations in several parameters in the environment. The aim of this study is to characterize and compare common physical parameters in the ordinary situation, where IVC-racks are kept in the same room with open cages. The cage type used was a polysulfone solid bottom cage. The parameters measured in this study were: illumination, temperature, relative humidity (RH) and acoustic level in both IVCs and open top cages. No animals were in the cages during light intensity, but there was bedding in the cage during acoustic measurements and both bedding as well as a half-full food hopper during the illumination measurements. The temperature and (RH) measurements were carried out with three male rats in each cage. There were differences between IVCs and open top cages in all measured parameters. The light intensity was lower in IVCs, most likely due to more compact cage placement in the rack and the additional plastic cover lid of the cage. Both maximum and minimum temperatures were 1-4 °C higher in IVCs; which suggests that their ventilation is incapable of taking away heat, produced inside the cage. Similarly, the relative humidity was higher in the IVCs. The sound level adjusted to rat's hearing with R-weighting was higher in IVCs when compared to open cages. Furthermore, the sound level was highest in the corners next to the ventilation valves. In conclusion, there may be differences between open cages with IVCs involving several physical parameters of cage environment and this may confound comparisons between results obtained in these cage systems.

Introduction

New caging and innovative items are being introduced to provide a more structured environment within the cage. Many of these innovations cannot be seen as 'pure' or single procedures, but rather as a mixed exposure with a multitude of operant factors, possibly having an impact on animals and research. One of those new kinds of caging systems is the individually ventilated cage (IVC), where each cage receives its own filtered air flow, primarily designed for health status maintenance and occupational
safety. Other potential benefits include: protection of small groups of animals against infections, protection of the environment from the animals and compensation for poor air change rates in the room (Brandstetter et al., 2004). Even though the cages may be the same as those used in open cage systems, the physical environment inside the cage may not be identical.

Reports on IVC-systems in the scientific journals can be divided into those concerned with the design and recommendations for (Höglund & Renström et al., 2001; Renström et al., 2001; Hawkins et al., 2003; Brandstetter et al., 2005) and characterization of the IVC environment (Krohn et al., 2003; Clough et al., 1995).

The move from traditional open cages to IVCs is bound to change the physical environment of the animals living in; what we have called, the “shoe-box”. It could be anticipated that at least temperature, relative humidity (RH), acoustic environment and light intensity may change in this transition. Traditionally in biomedical research, attempts are made to assess the individual effects of compounds and procedures and, usually, evaluation of many simultaneous events and their combinations are avoided. The term used here is standardization, and emphasis is on the fact that all other items are exactly the same between study and control groups. Comparison of open-top cages and IVCs without characterization of the physical environment may not reveal a single causative feature, because the change inevitably involves a mixed exposure. The aim of this study is to characterize and compare physical parameters in a common situation, where IVC-racks are housed in the same room with open cages.

**Materials and Methods**

**Animal room**

All the cages were kept in the same room (length x width x height; 5.5 x 3.5 x 3.0 m) along opposite walls. The locations of cage racks, room furniture, fluorescent tubes, air inlet and outlet are illustrated in Figure 1. The IVC-rack (Figure 2) included 20, while the open cages (Figure 3) were in two racks, ten cages each. The height of the open cage rack was 176 cm and that of IVC-rack was 186 cm.

![Figure 1. Layout of the animal room.](image-url)
Cage types

The measurements were done from two different caging systems: open top cages and individually ventilated cages (IVC). Cages made of polysulfone (Tecniplast, Buguggiate, Italy, type1500U, dimensions 48.0 x 37.5 x 21.0 cm) with a solid bottom were used. Both cage types had a stainless steel wire lid, while in the IVCs there was an additional polysulfone cover, which contained the air supply and the exhaust air valves and a passive filter at the top of the cover. This filter allows gas exchange for a short period, when the cage is not docked to the IVC-system.

The IVC-system consisted of a ventilation module, which had both supply and exhaust units (Slim Line™, Tecniplast, Buguggiate, Italy) and the individually ventilated cage rack (Sealsafe™, Tecniplast, Buguggiate, Italy). The supply unit delivered HEPA (High Efficiency Particulate Air) filtered air into the cage, taken from the room itself, separately through each cage’s supply valve, at the cage cover end. The exhaust air was taken out from the cages, also at the same end, through the exhaust valve and voided back to the room through a three filter set down to HEPA level.

Illumination

Artificial lights with two fluorescent tubes (light color warm white) were on from 06.00 to 18.00; their location in the room (106 cm below the ceiling) is illustrated in Figure 1. The IVC-rack had a stainless steel shield on top, and the open cages were covered with black plastic sheeting to prevent direct light entering the cages. No animals were in the cages during the light intensity measurements, but there was bedding in the cage and the food hopper was half full. The illuminometer (Roline digital lux meter RO 1332, Rotronic AG, Bassersdorf, Switzerland) was placed at the center of the cage floor on the bedding layer and single measurements were taken from both IVCs (Figure 2) and open cages (Figure 3).

Acoustic environment

A sound analyzer (Norsonic 121, Norsonic AS, Lierskogen, Norway) was used for noise measurement. The measurement system was calibrated using a sound level calibrator (Wärtsilä model 5274, MIP Electronics Oy, Kerava, Finland). Four cages from both cage types were measured four times, once from each corner. The cages had bedding, but no animals were inside the cages during the measurements. Measurements were taken from cages marked with ⚫ as shown in Figures 2 and 3. Equal sound pressure levels for one minute (Leq, 1min) in third-octave bands between 31.5 Hz and 20 000 Hz were measured with 1/2 an inch condenser microphone (Norsonic 1225, Norsonic AS, Lierskogen, Norway.). The microphone was placed about 5 cm from the walls and the bottom of the cage, and directed towards the corner of the cage.

In the weighted equal sound pressure level calculations, R-weighting and A-weighting were used. The

Figure 2. Frontal view of cage and ventilation unit location in IVC-cage rack with cage-specific illumination values (lux) and cages where acoustic, temperature and humidity measurements were done.
basis of the R-weighting for rat hearing sensitivity and the numerical values are described in detail by Björk et al., (2000) and Voipio (1997). The A-weighting is commonly used in human sound experiments. The total weighted equal sound pressure levels were computed summing the weighted third-octave band levels on the energy bases. The weighted equal sound pressure level in each cage was computed as the mean value in the four corners on the energy bases.

**Temperature and RH**

Temperature and (RH) measurements were carried out with animals in the cages. Fischer344 (F344/NHsd, Harlan, Horst, The Netherlands) male rats were used in this study. The rats were 40 weeks old and weighed 380 - 400 g, three animals per cage. The cage floors were covered with 3.0 l aspen chip bedding (size 4 x 4 x 1 mm, 4HP, Tapvei Oy, Kaavi, Finland).

Municipal tap water was provided in polycarbonate bottles with stainless steel drinking nipples, changed once a week and refilled once in between. Irradiated pelleted (25 kGy) feed (2016 Global Rodent Maintenance, Teklad, Bicester, UK) was given ad libitum, added once a week. Temperature and RH were measured with Besser® 7009032 Wireless Weather Station and with two Techno line TX4 433 MHz sensors (Besser, Borken, Germany). Measurements were taken simultaneously from both cage types (IVC and open top) and from the room for 7 days. The sensors were placed inside the feed hopper next to the pellets. Readings were taken once a day to provide minimum and maximum values over the previous 24 h period for

![Table plate]

**Figure 3.** Frontal view of cages in open cage racks with cage-specific illumination values (lux) and cages where acoustic, temperature and humidity measurements were done.
temperature and RH. Measurements were taken from cages as marked in Figures 2 and 3.

Results
Illumination
The light intensity at 1 m above floor in the open cages was 16-18 lux compared to 6–9 lux in the IVC’s, with upper cage rows showing considerably higher values. The light intensities of IVC-racks were lower than those measured in the open cages at corresponding levels. More detailed, cage specific, values are shown in Figures 2 and 3.

Acoustic environment
The sound level adjusted with R-weighting in empty IVC cages was 20-25 dB(R) compared to 12-18 dB(R) in the empty open cages, with the corresponding adjusted A-weighting being 45-47 dB(A) and 46-49 dB(A), respectively. The sound level was less in the open cages in the lower shelves of the rack, while in the IVCs the front of the cages showed higher sound levels compared to the back corners in the vicinity of the air valves. The sound frequency in both cage types was 16 – 16000 Hz. The mean sound pressure levels in the third-octave bands between 31.5 Hz and 20000 Hz on the energy bases of both cage types with R- and A-weighting and un-weighted (lin) are shown in Figures 5a and 5b.

Temperature and RH
There was a marked difference in temperature and RH between inside the IVC’s and open top cages when compared to both open top cage and room values. In the IVCs, the maximum and minimum temperature values in the IVCs were 1-4 °C higher than the room temperature. In the open top cages the temperatures were at the same level as the room temperatures. There was a similar tendency noted in RH as shown in Figure 4.

Figure 4. Deviation in the 24 hourly single cage maximum and minimum values inside the cage for temperature and relative humidity from the corresponding room values during the week and between cage changes. A and C are values for IVC-cages and B and D for open cages. Cage changes are marked with an arrow.
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Discussion

IVCs are a new isolation system being installed in a large number of laboratory animal facilities. The system provides protection to the animals against infections, and has clear occupational benefits to personnel, especially in leading to a reduction in the levels of airborne allergens (Renström et al., 2001). It is often assumed that the physical environment is the same in open cages and IVCs, when they are kept in the same room. Surprisingly it was found that this is not the case, and furthermore that the magnitude of the changes in physical environment is great enough that it could have an impact on the animals housed in these cages. Therefore it was decided to assess a set of easily measurable physical parameters in both caging systems.

The light intensity in IVCs was 10-60 lux lower than the corresponding values in open top cages. In the IVCs, the illumination varied between 3.8-28.9 lux and in the open top cages between 14.2-91.0 lux. The brightest cages were on the top row of both racks and the dimmest cages on the bottom row. Clough et al., (1995) has shown similar results in the transparent, polycarbonate positive individually ventilated (PIV)-cages but in the translucent, polypropylene control cages the illumination was much brighter than used in our experiment. This difference in results may be due to the black plastic sheeting that was placed on top of the open top cage racks to equalize the lighting in both cage types. It appears that in some open top cages at the highest level, in contrast to the situation in the IVCs, the lighting was too bright, and even exceeded values shown to cause retinal damage in albino rats (Stotzer et al., 1970; Weisse et al., 1974).

Sound spectra (Figures 5a and 5b) show decreasing sound levels when approaching 16000 Hz and consequently it can be assumed that no ultrasound exists in either cage type. Accordingly the measured R-weighted sound pressure levels depict the correct sound level which would be heard by the rats. In IVCs, the R-weighted sound levels were about 7 dB(R) higher than in open top cages; energywise the difference was five fold and in terms of loudness almost twice as great. Although the difference in sound levels audible to the rat (R-weighted) is large, it remains to be determined whether the levels measured (< 25 dB(R)) have any major impact on the animals.

Scheer et al., state the obvious: The climatic conditions in the cage are dependent on those of the surrounding room as well as the air supply of the cage-rack. In this study the temperature and RH in IVCs were the same as in the animal room when measured without animals, but placement of animals into the IVCs increased the temperature by 3-4 °C and RH by about 6 % in these cages.

In the open top cages, the temperature and RH were at the same level as in the animal room. This is in agreement with the results of Clough et al. (1995) who have shown similar results in PIV-cages. It appears that IVC-ventilation is unable to remove all the heat produced in the cage. Potential sources are heat from the animals as well as heat generated from urine-feces-bedding fermentation. Heat emission for three animals in a cage is estimated to be...
about 12 W (Heine, 1998). However, fermentation reactions are unlikely to occur because ventilation tends to keep bedding too dry.

It appears that the transition from traditional open top cages to IVCs may lead to changes in the physical environment. This makes any comparisons of these caging systems problematic without characterization of the physical parameters e.g. lighting intensity, sounds, temperature and RH. In conclusion, comparison of open cages with IVCs involves several physical parameters in the cage environment, which may confound straightforward comparisons.

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References


CHAPTER III

Work for food – A solution to restricted food intake in group housed rats?

Work for Food – A Solution to Restricting Food Intake in Group Housed Rats?

by Niina Kemppinen1,*, Anna Meller1, Kari Mauranen1, Tarja Kohila1 & Timo Nevalainen1,4

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Summary
Rodents spend a great proportion of their time searching for food. The foraging drive in rats is so strong that the animals readily work for food even when food is freely available. Commonly used ad libitum feeding is associated with a reduced life span, increased incidence of tumours and risk of liver and kidney diseases. It is also considered to be the most poorly controlled variable in rodent bioassays. The aim of this study was to assess whether rats will gnaw wood in order to obtain food hidden in wooden walls, whether this activity has a beneficial effect on controlling weight gain, and whether a typical diurnal activity rhythm is maintained. A total of 18 BN/RijHsd and 18 F344/NHsd male rats were housed in either open or individually ventilated cages (IVC), three rats in each cage. 10 of 36 were fitted with a telemetric transponder. Four groups were used: two groups (diet board and plain board) with a maze made of two crossed aspen boards, the third having a rectangular aspen tube. One maze was of plainboard, but the other included drilled holes snugly loaded with food pellets, the “diet board”, such that the rats had to gnaw wood to reach the food. The other two groups – and the controls – were fed ad libitum. The study used a crossover design and the added item was changed every two weeks. Rats, added items, and amount of food left at the end of the two week period were weighed. The statistical assessment showed that in terms of weight gain there was a significant interaction both in IVC- (p = 0.005) and in open cages (p < 0.001) between the strains and the group. In the F344 rats the diet board was more effective in controlling weight, but when combining the strains, all comparisons with diet board were significant (p < 0.05). Use of strain and added item as main effects, and age as covariate, showed that in the IVC-system there was a significant (p < 0.001) interaction between the strain and the group, this effect being rather clear in the F344 rats in terms of amount of food disappearing. In the open cage system, both strain and group were significant (p < 0.001) factors; all three comparisons with diet board were significant (p < 0.001) in the amount of food disappearing. In conclusion, the work-for-food approach appears to be a promising way of avoiding obesity without causing untoward effects on diurnal activity in rats. Hence, the approach may have considerable refinement and reduction potential.

Introduction
Obesity resulting from overeating is a universal problem; and restricted feeding is the best remedy to cure obesity-associated problems. This is also true in laboratory animals. Laboratory rodents are commonly fed ad libitum, e.g. food is available all the time. However, there is ample evidence that ad libitum feeding increases the incidence of kidney, heart diseases, and neoplasias and shortens lifespan in rats (Roe, 1994; Roe et al., 1995; Hubert et al., 2000).

Keenan et al. (1999) has stated that ad libitum feeding of rodents is the most poorly controlled experimental factor in animal-based research. In the long-
term, studies rats die prematurely due to malignancies and degenerative diseases, and this impairs the statistical sensitivity of the study and leads to more animals being needed.

Group housing is the preferred method, and indeed this is a regulatory requirement in Europe (Council of Europe 2007; European Union 2007). However, when animals are group housed, there is no practical or effective way to restrict evenly the food intake of all individuals within the group. Food consumption within the group may also vary, with the dominant animal eating more than the others. When animals are housed individually, restricted feeding is technically possible, but it may, depending how and when food is offered, change the diurnal rhythm. Furthermore, solitary housing is not practical because it requires more cages, and hence is costly. Rats are nocturnal animals and in their natural environment they forage for food and eat mainly during the dark phase because there is less risk posed by predators. In animal facilities, rats also eat predominantly during the dark period when the food is available ad libitum (Spiteri, 1982; Strubbe et al., 1986; Strubbe & Alingh Prins, 1986), in fact eating during the dark is probably genetically determined (Ritskes-Hoitinga & Strubbe, 2004). It has been shown that when ad libitum feeding was reinstated after a restricted feeding schedule, the rats will immediately revert to their original feeding pattern (Spiteri, 1982; Strubbe et al., 1986). Locomotion behaviour also increases if the food deprivation period is longer than six hours (Vermeulen et al., 1997); a probable consequence of food searching behaviour.

Daily feeding activity and other diurnal rhythms are controlled by the circadian oscillator, which is located in the suprachiasmatic nuclei in the hypothalamus (Stephan, 1984; Strubbe, et al., 1987; Ritskes-Hoitinga & Chwalibog, 2003). When rats are fed with restricted feeding they have access to food for a few hours, and in most cases this coincides with the housing facility’s working hours. In this kind of situation, they eat all the food immediately, which will impair both natural feeding patterns and gastrointestinal physiology. This can lead to a phase-shift of many biochemical and physiological functions in the gastrointestinal tract of nocturnally active rodents and further changes in serum insulin and glucose (Strubbe & Alingh Prins, 1986; Strubbe, 1987; Rubin et al., 1988), mucosal enzymes of small intestine (Saito et al., 1975) and bile flow (Ho & Drummond, 1975) in rats. Moreover, it has also been shown that an altered feeding schedule results in changes of blood pressure, heart rate and behavioural activity of rats (van den Buuse, 1999).

A decrease in rat food intake in the early studies was achieved with meal feeding; i.e. rats had access to food for only couple of hours a day (Saito et al., 1975; Stephan, 1984; Strubbe, & Alingh Prins, 1986; Roe et al., 1995; van den Buuse, 1999), or simply offering them a certain amount of food (Vermeulen et al., 1997; Markowska, 1999; Hubert et al., 2000). However, these methods necessitate solitary housing of rats.

There are studies trying to combine group housing and restricted feeding. Johnson et al. (2004) covered the feeding area except for a one cm wide slot, where the food was available to the rats. In the same study they also had a “foraging device”, where rats had to work, i.e. to move gravel for access to food. With the slot approach the rats spent more time feeding but consumed less food and with no effect on body weight. The rats preferred eating from the “foraging device”, and though they had to work for food, the body weights of these rats were even significantly higher than in ad libitum fed controls. A third approach that had been tried is the addition of largely indigestible sugar beet pulp fibre to the chow; there were reduced weight gain benefits, but also enlarged GI-track - especially caecum - in the increased fibre-fed group (Eller et al., 2004).

We hypothesized that rats will only work - in this case gnaw wood - for food they necessarily need, provided that the work intensity is correctly set. The aim of this study was to assess whether a novel system of food restriction would have any effect on weight gain over a short period, food utilisation and
amount of wood gnawed in adult rats and whether their time budget differs from ad libitum fed rats.

Materials and Methods

Animals

A total of 18 BN (BN/RijHsd) and 18 Fischer344 (F344/NHsd) male rats, all supplied from Harlan, (Horst, The Netherlands), were used in this study. 10 of which were fitted with a telemetric transponder (details below). The rats were 25 weeks old and weighed 280 - 370 g (BN) or 350 - 460 g (F344), respectively, at the beginning of the experiment.

Animal housing and care

Rats were housed in the same room either in open top polysulfone cages (Tecniplast, Buguggiate, Italy) or polysulfone individually ventilated cages (IVC) (Tecniplast, Buguggiate, Italy) (3 rats / cage). The cage type used was 1500U Eurostandard IV S (48.0 x 37.5 x 21.0 cm – floor area 1500 cm$^2$) with a solid bottom and stainless steel wire lid; IVC cages had their own double lids. The cage floor was covered with 3.0 l aspen chip bedding (of size 4 x 4 x 1 mm, 4HP, Tapvei Oy, Kaavi, Finland). The cages were changed weekly. The room temperature was 21.2 ± 0.3°C and relative humidity (RH) 53.5 ± 7.7 %, but the temperature was 1 – 4 °C and RH 2 – 3 % higher in the IVCs than both in open cages and in the room. Artificial lighting with fluorescent tubes (light colour warm white) were on from 06.00 to 18.00 and the light intensity at 1 m above floor in the open cages was 16-18 lx compared to 6–9 lx in the IVC’s. The sound level adjusted with R-weighting in empty IVC cages was 20-25 dB(R) compared to 12-18 dB(R) in the empty open cages, with the corresponding adjusted A-weighting being 45-47 dB(A) and 46-49 dB(A), respectively. Tap water was provided in polycarbonate bottles and changed once a week and refilled once in between. For a more thorough description, see Kemppinen et al. (2008) preceding paper.

Experimental procedure

Animals were housed three animals per cage, one of them with telemetric transponder. The experiment utilized a crossover design with two week rounds and a rotational order. Within both strains there were two different kinds of mazes (diet board and plain board) made of two crossed aspen boards (34.0 x 14.7 x 3.2 cm; 21.1 x 14.7 x 3.2 cm), a rectangular aspen tube (20.0 x 12.0 x 12.0 cm), or controls without any addition (Figure 1). One maze included holes for food pellets, the diet board, where rats had to gnaw for food, the other was of plain board. The items were made out of aspen because this was the same material as the bedding presumably with the same emissions.

Irradiated (25 kGy) pelleted feed (2016 Global Rodent Maintenance, Harlan Teklad, Bicester, UK) was offered to three groups (plain board, tube and control groups) ad libitum, while the diet board group had the food pellets embedded snugly in drilled holes (12 mm) of the aspen board. The feed was added once a week and weighed. The aspen boards were weighed before and after the food pellets were placed into the holes. These diet boards were changed once a week. After the change, the remaining food pellets were removed from the diet-boards and weighed. Rats were weighed before and
after every study round. All the aspen items were weighed before use and at cage change. In addition, to assess the effect of the various feeding regimens on the rats’ physiological activity and heart rate, ten rats had been implanted with a radio telemetry transmitter (model TA11PA-C40; Data Sciences International, St.Paul, MN, USA). The cylinder shape transmitter body (3.0 cm long, Ø 1.5 cm) monitored pressure and activity via a fluid filled catheter (8 cm long) for sending the signals to an electronics module. The electronics module translated the signals into digitized form and transmitted them to the receiver plate located under the cage. The receiver detected the transmitted signal and converted it to a form readable by the computer.

The rats were anesthetized with the combination of fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium) + midazolam (Dormicum®, Hoffmann - La Roche AG, Grenzach-Wyhlen, Germany)(0.15 - 0.20 ml/100g SC). The abdominal area was clipped and then scrubbed with MediScrub®, 1 % triclosan solution (Medichem International, Sevenoaks, UK) solution and disinfected with chlorhexidine solution (Klorohexol® 5 mg/ml, Leiras, Turku, Finland), and an ocular lubricant (Viscotears®, Novartis Healthcare, Copenhagen, Denmark) was applied on both corneas. A sterile drape was placed over the surgical area and a small area cut away to enable a 3 cm incision to be made through the skin along the abdominal midline. The sterile transmitter was pre-soaked in sterile saline for at least 20 min before the surgery and then placed into the abdominal cavity, and the catheter into the abdominal aorta. The transmitter was sutured into the abdominal wall with 4-0 Ethicon® Ethilon®II (Johnson & Johnson Intl, St-Stevens-Woluwe, Belgium) and the abdominal and skin incisions were closed with 5-0 Ethicon® Vicryl® (Johnson & Johnson Intl, St-Stevens-Woluwe, Belgium). After the surgery, the animals were given twice a day 0.01 – 0.05 mg/kg SC buprenorphine (Temgesic®; Schering-Plough Europe, Brussels, Belgium) and once a day a dose of 5 mg/kg SC carprofen (Rimadyl®; Vericore Ltd., Dundee, UK) and parenteral fluids for three days. The pain medication for each rat was titrated with individual response. All rats were given initially buprenorphine at the highest dose; this was continued for at least two days; and carprofen medication for at least three days. The animals were allowed to recover for ten days before the experiment was started.

Data processing and statistical analysis

Activity and heart rate were processed for time budget graphs from the telemetric signals for ten min periods on the first, third, seventh and 13th night and the following light period for each night for all instrumented rats. The number of ten minute periods without activity (activity = 0) were calculated from the graphs, and comparisons made between the groups during the 13th night, and between the days processed in the diet board and plain board group.

All data was assessed with Kolmogorov-Smirnov for normality of distribution. Mixed-model repeated measures ANOVA using strain and group as main effects and age as covariate was applied to weight, disappearance of food, wood gnawed and activity during the dark. Significance was set at p < 0.05.

Results

Calculation on a rat basis showed that with respect to the weight gain, there was a significant interaction both in IVC (p = 0.005) and in open cages (p < 0.001) between strain and group (Figures 2A & 2B). In F344 rats, the diet board was more effective in controlling weight, but when combining the strains, all comparisons with diet board were significant (p < 0.05). When the calculation was done on a cage basis, then it seemed that only the rats with the open-cage type diet board displayed any significantly (p = 0.008) reduced weight gain as compared to the plain board group.

In terms of food consumption and in the IVC-system, there was a significant (p < 0.001) interaction between strain and group, with the effect being
clear in F344 rats (Figures 3A and 3B). In the open cage system, both strain and group were significant \((p < 0.001)\) factors; in all three comparisons differences with diet board were significant \((p < 0.001)\). When the strains were pooled, the difference was between 12 - 18 % less food eaten as compared to respective controls.

The amount of wood gnawed differed significantly from normal distribution; hence a mixed model was applied to the ranks. In terms of the amount of wood gnawed, there was a significant \((p = 0.001 - 0.005)\) interaction between strain and group in both cage types. The rats gnawed more wood with diet board as compared to the plain board and tube groups in both caging systems. Furthermore, F344 rats gnawed wood more than BN rats (Figures 4A and 4B).

Typical activity and heart rate recordings for the last light and dark period of the two week round for both BN and F344 rats are shown in Figures 5A - 5D. Calculation from all diet board and plain board activities shows that in both cage types there was a significant interaction \((p < 0.001)\) between the
strain and light, with both of the strains being more active during the dark. F344 rats were significantly (p < 0.05) more active in the dark phase than BN rats in both groups. There were no differences in the activity of the rats between the diet board and plain board groups.

**Discussion**

It has been demonstrated that rats prefer to work for food. Carder & Berkowitz (1970) and Neuringer (1969) reported that even if the rats had free access to food they would rather earn their food as long as the work demands were low. In a preference test, rats preferred to eat mostly from the foraging device which required digging gravel to achieve access (Johnson et al., 2004). This preference of the rats may reflect their need to perform foraging behaviour as they would in their natural environment.

All the rats with the diet board grew less than other groups in both cage types; especially in the F344 rats the diet board was effective in controlling weight. The F344 rats lost weight in the diet board group especially in the open cages, when the rats were older, but the magnitude of loss was marginal – only a few grams over two weeks, most likely fat tissue (Figure 2). The working hypothesis has been that rats should grow less on the restricted feeding (Roe et al., 1995; Hubert et al., 2000), but this has not been observed in all studies. With the “foraging device”, the weight gain of the rats was higher than in ad libitum fed controls; and when the rats had limited access to food, their body weights remained unchanged, both being indications that the approach had been unsuccessful (Johnson et al., 2004).

Eller et al. (2004) have tried to determine whether consuming sugar beet pulp fibre made from water-soluble polysaccharides would have any effect on the weight gain of rats. The rats indeed grew less with the fibre diet, but autopsy after the study revealed an enlarged digestive system in the rats that had received the fibre enriched diet – especially the caecum was enlarged. This may be attributa-
Figure 5. Typical single rat (A = BN – dark, B = BN – light, C = F344 – dark, D = F344 - light) activity and heart rate (HR) recording for the last 24 h of the two week round. There was a significant interaction (p = 0.000) between the strain and light, and both strains were more active during the dark. F344 rats were significantly (p < 0.05) more active in the dark phase than BN rats.
ble to the hygroscopic effect of the fibre. The F344 rats ate more than the BN rats in all of the groups. In the open cage system rats ate significantly less in the diet board group compared to the other three study groups. When the strain specific data was pooled the difference was between 12 - 18 % less as compared to the respective controls. The rats in the open cages ate more in the plain board group than in the two other control groups – apparently because the plain board round followed the diet board round, and the animals regained their weight loss in that round (Figure 3). In the study of Johnson et al. (2004) the rats consumed less food when they had limited access to food, while the contrary was true with the “foraging device”, both as compared to controls. The rats gnawed the wood most with the diet board as compared to plain board and tube groups in both cage types. This was an unavoidable task if they wished to eat the food pellets. The F344 rats gnawed wood significantly more than the BN rats - this may relate to a difference in the natural behaviour of these two rat strains (Figure 4). Eskola et al. (1999) have shown that rats would spontaneously gnaw aspen blocks and tubes but this opportunity for gnawing combined with ad libitum feeding had no effect on the growth of Wistar rats, a situation similar to F344 rats in plain board and tube groups. The F344 rats were significantly more active during the dark than the BN rats in both cage systems. There were no differences in the activity between the plain board and diet board groups suggesting that working for food was not overly strenuous to the rats. Furthermore, the activity of the rats at that time did not differ from their activity during ad libitum feeding. It has been shown that when rats have limited access to food they spend more time feeding, but with the “foraging device” the time spent feeding was markedly decreased. There were only negligible changes between the study groups in their relative total activity levels (Hawkins et al., 1999; Johnson et al., 2004). There were no changes in the social hierarchy of the rats and no increased fighting or stereotype behaviour when rats had limited access to food (Hawkins et al., 1998). The rats eat most of their food in the dark. In the study of the Spiteri (1982) the rats consumed 94 % of their food intake during the dark. The normal feeding activity of rats consists of two peaks during the dark, the first one at the beginning of the dark phase and the other at the end (Spiteri, 1982; Strubbe et al., 1986a). Light is a strong ‘Zeitgeber’ because it shifts the clock in a circadian time-dependent way ensuring synchrony with the external light-dark cycle. The feeding activity and other diurnal rhythms are controlled by the circadian oscillator of the suprachiasmatic nuclei in the hypothalamus. It has been claimed that there are more oscillators involved in the circadian system and this provides the flexibility needed for adaptation to different external and internal stimuli (Anglés-Pujolrás et al., 2006). When the rats are given access to meals at set times for a few hours each day, they eat all the food almost instantaneously and spend the rest of the day without food; this impairs their natural feeding activity and associated gastrointestinal physiology. This study used the diet board for food restriction allowing rats to enjoy a natural feeding pattern and indeed feeding activity was similar to the plain board group (Figure 5).

The diet-board has clear advantages over previous methods of restricted feeding. The rats can eat at any time and in addition is unlikely to alter biochemical and physiological phenomena timed by circadian rhythm, as opposed to set meal times and most other, if not all, restricted feeding methods (Ho & Drummond, 1975; Saito et al., 1975; Stephan, 1984; Strubbe et al., 1986b; Strubbe et al., 1987; Rubin et al., 1988; van den Buuse, 1999). We conclude that the diet board seems to be a promising way to control obesity and health problems in laboratory rats. Challenging questions still need to be answered to determine whether this approach has refinement and reduction potential.
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CHAPTER IV

The effect of dividing walls, a tunnel and restricted feeding on cardiovascular responses to cage change and gavage in rats (*Rattus norvegicus*).


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The Effect of Dividing Walls, a Tunnel, and Restricted Feeding on Cardiovascular Responses to Cage Change and Gavage in Rats (Rattus norvegicus)

Niina M Kemppinen, Anna S Meller, Kari O Mauranen, Tarja T Kohila, and Timo O Nevalainen

Cage change and gavage are routine procedures in animal facilities, yet little is known about whether housing modifications change responses to these procedures. Telemetric activity and cardiovascular parameters were assessed in this experiment. BN and F344 male rats were housed in open or individually ventilated cages, each containing 3 rats, 1 of which had a transponder. A crossover design was used, in which 2 groups were given dividers made of 2 intersecting boards (1 form contained holes loaded with food pellets; the other did not) and 1 group was given a rectangular tunnel. On day 8 of each 2-wk period, the cages were changed; on day 11, rats were gavaged. The parameters were evaluated for the first hour and for the following 17 h. Baseline values for each rat were subtracted from the corresponding response values. The presence of objects did not affect the responses of F344 rats to cage changing or gavage. In BN rats with IVCs, the presence of the plain divider modified the response to both procedures. Responses to procedures appeared to be dependent on both the strain and the cage object, thus complicating the establishment of valid general recommendations.

Abbreviations: bpm, beats per minute; HR, heart rate; IVC, individually ventilated cage; MAP, mean arterial pressure.

The new European regulations on laboratory rodents mandate the provision of sufficient nest material to build a complete, covered nest or, if doing so is not possible, providing a nest box. Because rats are poor nest-builders, they must be provided with objects for this purpose. Moreover, objects that provide cover or divide the cage area may allow the rats to initiate or avoid contact with cagemates.

All these regulations are rather specific, but generally the same for all laboratory rodents, that is, rats, mice, gerbils, hamsters, and guinea pigs. However, all rodent species and even strains and stocks within a species may have different needs. These differences raise the question of whether general guidelines, which may be valid for 1 species, may have a negative effect on welfare in other species and strains.

Cage change is a frequent routine procedure in animal facilities that induces temporary, but significant, cardiovascular and behavioral changes in rats. Similarly, the frequency and time of changing, type of the bedding material, light intensity, and length of the dark period all modify the intensity of the response to cage changing. The effects on physiologic parameters, such as blood pressure and heart rate, after the cage change seem to be a consequence of the transfer procedure itself and of the novel environment.

Two features of rats suggest potential advantages of placing objects in the cage. First, rats are known to have a good sense of smell—1493 specific olfactory receptor genes have been identified on the cilia of the olfactory neurons—and smell is their primary sense for monitoring their environment. Second, rats have dominance hierarchies in which fighting is essentially territorial, rather than for any specific object. The term ‘skirmishing’ has been used to describe a pattern of behaviors often assumed to be aggressive in rats; in 1 study, the frequency of skirmishing was increased during the first 15 min after a cage change. Consequently, cage objects may retain a familiar odor cue during cage change; the presence of the old item in the new cage reduces the aggressive behavior of rats that is triggered by regrouping.

Gavage is a method widely used to administer test compounds into the stomach of laboratory rats. Rats display increased blood pressure and heart rate (HR) immediately after gavage, and these increases may persist for 30 to 60 min after the procedure. Furthermore, elevations in plasma corticosterone levels have been measured in rats after gavage. The selection of the correct administration volume is a suitable probe material important to performing this procedure properly, but whether housing can be advantageous is unknown.

Housing refinements have not been assessed in regard to their effect on refining the performance of procedures in rats, although housing modifications can alter their physiology and behavior. Rats have lower blood pressure and HR when housed in a grid or plastic floor. Rats also prefer a cage with shelter to a barren environment, perhaps because they prefer to spend most of the light phase inside the shelter. Rats with a furnished environment are more active than those which lack such objects, and the presence of a shelter in the cage decreases fearfulness. Finally, the availability of cage objects may allow the rats to exhibit species-specific behaviors.
In many previous studies of the effects of cage changing or gavage, the presence of objects in the cages was not described. However, I study of both Sprague-Dawley and spontaneously hypertensive male rats showed that providing a multifaceted enrichment program over a week did not affect HR or systolic blood pressure responses to placement in a standard rodent restrainer for 60 min. However, after removal from the restrainer, the rats showed a secondary increase in HR and systolic blood pressure that was significantly attenuated in enriched compared with nonenriched rats of both strains. Moreover, enriched rats of both strains had lower HR and systolic blood pressure responses to a variety of procedures, including removal of a cagemate, tail-vein injection, and exposure to the odor of urine and feces of stressed male or female rats.

We hypothesized that cage objects would alter the effect of cage change and gavage on telemetrically recorded cardiovascular parameters and locomotor activity. This study was designed to evaluate the effect of an aspen wall divider with or without restricted feeding and of the presence of an aspen tunnel in the cage on these measures after routine cage changing and gavage of laboratory rats in both open-top cages and IVCs.

Materials and Methods

The study was done in the Laboratory Animal Centre, University of Helsinki. The study protocol was reviewed and approved by the Animal Ethics Committee of the University of Helsinki.

Animals. A total of 12 BN (BN/RijHsd) and 12 Fischer 344 (F344/NHsd) male rats, all supplied from Harlan (Horst, The Netherlands) were used in this study. The rats were 25 wk old and weighed 280 to 370 g (BN) or 350 to 460 g (F344) at the beginning of the experiment.

Animal housing and care. For the first 8 wk, all rats were housed (3 rats per cage) in the same room in polycarbonate IVCs (Techniplast, Buguggiate, Italy) and then for 8 wk in open-top polycarbonate cages (Techniplast). The caging (48.0 × 37.5 × 21.0 cm; floor area, 1500 cm²) had a solid bottom and stainless steel wire lid; each IVC had its own double lid. The cage floor was covered with 3.0 L aspen chip bedding (4 × 4 × 1 mm; 4HP, Tapvei Oy, Kaavi, Finland). The cages were changed weekly.

The room temperature was 21.1 ± 0.3 °C and relative humidity was 53.5% ± 7.7%, but the temperature was 1 to 4 °C higher and relative humidity was 2% to 3% higher in IVCs than in open cages and the room.

Artificial lighting with fluorescent tubes (light color, warm white) was on from 0600 to 1800, and the light intensity at 1 m above floor in the open cages was 16 to 18 lx.

Cages and the room. Artificial lighting with fluorescent tubes (light color, warm white) was on from 0600 to 1800, and the light intensity at 1 m above floor in the open cages was 16 to 18 lx.

Cage maintenance, Harlan Teklad, Bicester, UK) was offered to 3 groups (plain board, tunnel, and control groups) ad libitum, whereas the diet board group had the food pellets embedded snugly into drilled holes (12 mm) in the aspen board. The diet board reduces food consumption by 12% to 18% in both F344 and BN rats. The transition to the diet board was carried out without acclimation, as for the other items.

Eight rats, 4 BN and 4 F344, were implanted with radiotelemetry transmitters (model TA11PA-C40, Data Sciences International, St Paul, MN). The cylinder-shaped transmitter body (length, 3.0 cm; diameter, 1.5 cm) monitors blood pressure and locomotor activity by means of a fluid-filled catheter (length, 8 cm), which transmits signals to the electronic control module. The electronic module translates the signals into a digitized form and transmits them to the receiver plate located under the cage. The receiver detects the transmitted signal and converts it to a computer-readable form.

For implantation of the transmitters, the rats were anesthetized with the combination of fentanyl-fluanisone [Hypnorm (fentanyl citrate, 0.315 mg/mL; fluanisone, 10 mg/mL), Janssen Pharmaceutica, Beerse, Belgium] and midazolam [Dormicum (midazolam, 5 mg/mL), Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany] at a dose of 0.15 to 0.20 mL per 100 g SC (1 part Hypnorm, 1 part Dormicum, and 2 parts sterile water).

The abdominal area was clipped, scrubbed with 1% triclosan solution (MedisRub, Medichem International, Sevenoaks, UK), and disinfected with chlorhexidine solution (5 mg/mL; Klorhexol, Leiras, Turku, Finland), and an ocular lubricant (Viscotears, Novartis Healthcare, Copenhagen, Denmark) was applied on both corneas. A sterile drape was placed over the surgical area, and a small area was cut away to enable a 3 cm incision to be made through the skin along the abdominal midline. The sterile transmitter was presoaked in sterile saline for at least 20 min before surgery and then placed into the abdominal cavity; the catheter was inserted into the abdominal aorta. The transmitter was sutured onto the abdominal wall with 4-0 absorbable suture (Ethilon II, Johnson and Johnson International, St Stevens Woluwé, Belgium) and the abdominal and skin incisions were closed with 5-0 suture (Vicryl, Johnson and Johnson International).

After the surgery, the animals were given buprenorphine twice daily (0.01 to 0.05 mg/kg SC; Temgesic, Schering–Plough Europe, Brussels, Belgium), carprofen daily (5 mg/kg SC; Rimadyl, Vericore, Dundee, UK), and parenteral fluids for 3 d. The pain medication for each rat was titrated according to individual response. All rats initially were given buprenorphine at the highest dose for at least 2 d and carprofen medication for at least 3 d. The animals were allowed to recover for 10 d before the experiment was started.

After 1 wk of each 2-wk period, the rats were transferred to a clean cage between 11:00 and 13:00 by lifting the rats by the body with encircled fingers. Cage cleaning involved replacing the cage, all of the bedding and the water bottle; the cage lid and feed were retained. The tunnel and plain board were moved to the new cage, the diet board was changed. In the ad libitum feeding groups, more feed was added after weighing.
The implanted rats were gavaged 3 d after cage change between 09:00 and 10:00 h; they were lifted from the cage gently by grasping the chest. The grip was changed to the scruff of the neck when the rats were in the holder’s lap. The rat was gently stroked twice from chin to the base of the tail, and a stainless steel gavage tube (length, 84 mm; shaft diameter, 1.2 mm) was passed through the esophagus into the stomach and maintained in that position for 3 s before being retracted slowly; nothing was administered. The time line of the study is illustrated in Figure 2.

Data processing and statistical analysis. Means of locomotor activity, mean arterial pressure (MAP), and HR were processed at 5-min periods for the first hour after the procedure and for 17 h thereafter at 30-min periods, separately for the light and dark periods and for the 2 cage types. Mean baseline values of MAP and HR for each rat for the dark and light periods and both cage types were calculated from recordings obtained on day 7, the day before the procedures; these values were subtracted from the corresponding response values. Mixed-model repeated measures ANOVA (SPSS Windows, version 14.0, SPSS, Chicago, IL, USA) was used to assess means and parameter responses, and Bonferroni correction was used for posthoc comparisons. For activity calculation, group was used as a main effect and age as the covariate. For the MAP and HR calculation, activity and parameter baseline values were added into the covariates.

Figure 1. Study groups. (A) Diet board. (B) Plain board. (C) Tunnel. (D) Control. Both rat strains (BN and F344) in both IVCs and open cages had an additional object for 2 wk.
subsequent dark phase in the IVCs, the plain board BN group expressed a significantly ($P < 0.01$) smaller MAP response than did the control group, and in the open-top cages, the tunnel group exhibited a significantly ($P < 0.05$) lower MAP response than did control animals (Figure 4 C).

F344 rats and HR response. In both cage types of F344 rats, no differences HR response were seen before the dark period (Figure 5 A, B). The HR response of IVC F344 rats during the first dark phase was significantly ($P < 0.05$) lower in those exposed to the plain board as compared with the diet board. In open-top cages, the HR response was significantly decreased in F344 rats with the diet board in comparison with control rats.

Results

Cage change. The locomotor activity response during the first hour after IVC cage change was higher ($P < 0.001$) in the tunnel group of F344 rats than in the control and in the plain board groups (Figure 3A). In the open-top cages during the first dark period, the F344 rats in the plain board group were significantly less active as compared with both control ($P < 0.01$) and tunnel ($P < 0.05$) groups (Figure 3C). Overall, the locomotor activity response decreased ($P < 0.01$) in both strains after the first hour (Figure 3). MAP and HR response durations exhibited no differences between the cage items. The night–day difference values for the MAP and HR in the control group before the procedures are illustrated in Table 1.

F344 rats and MAP response. In the IVCs, the F344 rats displayed a significant ($P < 0.001$) difference between item MAP responses only during the subsequent dark period, during which the MAP of the diet board group was higher than that of the controls and the other 2 groups (Figure 4C). In the open cages during the first hour, the MAP responses experienced by the plain board F344 rats were significantly higher than those of the control group ($P < 0.05$) and the tunnel group ($P < 0.01$; Figure 4 A). Later, during the dark period, the MAP response was lower in the diet board group compared with the control ($P < 0.05$) and plain board ($P < 0.01$) F344 groups (Figure 4 C).

BN rats and MAP response. No differences between groups of BN rats were detected in either cage type during the first 60 min after cage change (Figure 4 A). During the remaining light period in the IVCs, the BN tunnel group displayed a significantly larger MAP response than did the control ($P < 0.001$) and diet board ($P < 0.05$) groups. In the open-top cages, the diet board BN animals exhibited a significantly ($P < 0.01$) higher MAP response compared with controls (Figure 4 B). During the subsequent dark phase in the IVCs, the plain board BN group expressed a significantly ($P < 0.01$) smaller MAP response than did the control group, and in the open-top cages, the tunnel group exhibited a significantly ($P < 0.05$) lower MAP response than did control animals (Figure 4 C).

F344 rats and HR response. In both cage types of F344 rats, no differences HR response were seen before the dark period (Figure 5 A, B). The HR response of IVC F344 rats during the first dark phase was significantly ($P < 0.05$) lower in those exposed to the plain board as compared with the diet board. In open-top cages, the HR response was significantly decreased in F344 rats with the diet board in comparison with control rats.
Cardiovascular effects of cage objects and procedures in rats

BN rats and HR response. Regardless of cage type, no differences in HR response between groups of BN rats were detected during the first hour or the first dark period after the cage change (P < 0.05) and the plain board (P < 0.001) and tunnel (P < 0.05) groups (Figure 5 C).

Table 1. The night and day baseline values and night–day differences of MAP (mmHg) and HR (bpm) of control F344 and BN rats in 2 different cage types (IVC and open top).

<table>
<thead>
<tr>
<th></th>
<th>F344 Night</th>
<th>F344 Day</th>
<th>BN Night</th>
<th>BN Day</th>
<th>Night – day</th>
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<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>115.3</td>
<td>108.9</td>
<td>93.8</td>
<td>92.0</td>
<td>6.4</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>376.4</td>
<td>320.9</td>
<td>309.0</td>
<td>277.6</td>
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</tr>
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**Figure 4.** MAP response (change ± SEM) of rats to cage change in IVCs and open-top cages. (A) First hour after cage change. (B) From 1 h after cage change to start of dark period. (C) First dark period after cage change. The y axis scale is the same in all figures. Overall there are 16 experimental units, 4 animals in 4 rounds. *, P < 0.05; #, P < 0.01; §, P < 0.001.

**Figure 5.** HR response (change ± SEM) of rats to cage change in IVCs and open-top cages. (A) First hour after cage change. (B) From 1 h after cage change to start of dark period. (C) First dark period after cage change. The y axis scale is the same in all figures. Overall there are 16 experimental units, 4 animals in 4 rounds. *, P < 0.05; #, P < 0.01; §, P < 0.001.

**BN rats and HR response.** Regardless of cage type, no differences in HR response between groups of BN rats were detected during the first hour or the first dark period after the cage change.
(Figure 5 A, C). During the period after the first hour until the start of the dark period in IVCs, there was a significantly ($P < 0.05$) higher HR response in BN rats with the tunnel compared with the diet board group (Figure 5B).

**IG-gavage**

Locomotor activity response to gavage did not differ between groups of both strains in both cage types until the first dark period, when the F344 rats in IVCs with plain boards were significantly more active than control rats ($P < 0.01$) and those with diet boards ($P < 0.05$; Figure 6 A). The locomotor activity response in both strains and cage types diminished ($P < 0.001$) after the first hour (Figure 6). There were no significant differences in the durations of the MAP and HR responses.

**F344 rats and MAP response.** The F344 rats living in open-top cages and with access to the tunnels had a significantly smaller MAP response during the first hour after gavage than did control ($P < 0.01$) and plain board ($P < 0.001$) groups, whereas from that point until dark, the plain board group exhibited a higher ($P < 0.001$) MAP response compared with those of all other groups, including the controls (Figure 7 A, B). During the subsequent dark phase, F344 rats housed in IVCs with diet boards or plain boards showed smaller ($P < 0.001$ for both groups) MAP responses than that of the control group, but in open-top cages, the F344 rats in the tunnel group had a lower ($P < 0.05$) response than did control and plain board rats (Figure 7 C).

**BN rats and MAP response.** BN rats in IVCs experienced a lower ($P < 0.01$) MAP response when the plain board was in the cage during the first hour after gavage, compared with controls. During the same period, the BN rats living in open-top cages with tunnels had a significantly lower MAP response than did control rats ($P < 0.05$) or those with diet boards ($P < 0.01$; Figure 7 A). During the subsequent light period (before dark), the MAP response in the IVC plain board group was decreased compared with those of control ($P < 0.05$), diet board ($P < 0.01$) and tunnel ($P < 0.001$) groups of BN rats. BN rats in open-top cages with plain boards displayed significantly ($P < 0.01$) greater MAP responses than did those with tunnels (Figure 7 B). During the dark in IVCs, the response differences disappeared, but in open-top cages, the MAP responses of the plain board group of BN rats were still significantly greater than those in control ($P < 0.05$), tunnel ($P = 0.01$), and diet board ($P < 0.001$) groups (Figure 7 C).

**F344 rats and HR response.** Significant response differences during the first hour after gavage were seen only in rats in IVCs; that is, HR response was significantly ($P < 0.01$) lower in the tunnel group compared with controls (Figure 8 A). During the remainder of the light period in open-top cages, the plain board group displayed a significantly ($P < 0.001$ for all comparisons) larger HR response compared with that of controls, rats with diet boards, and with rats with tunnels (Figure 8 B). During the subsequent dark phase, groups exposed to the diet board ($P < 0.01$) and plain board ($P < 0.001$) in IVCs had decreased HR responses when compared with those of controls, and in open-top cages, the plain board group of F344 rats showed a significantly higher HR response than did the diet board ($P < 0.05$) and tunnel ($P < 0.01$) groups (Figure 8 C).

**BN rats and HR response.** During the first hour after gavage, the BN rats in IVCs with tunnels or plain boards had smaller ($P < 0.01$ for both comparisons) HR response than did controls, whereas in open-top cages, the diet board group had higher HR responses than did controls ($P < 0.05$) and the tunnel group ($P < 0.01$; Figure 8 A). During the remaining light period, the plain board group of BN rats in IVCs displayed a significantly ($P < 0.05$) lower HR response than did controls, whereas in open-top cages, the BN rats in the tunnel group exhibited a significantly ($P < 0.05$) lower response compared with those of the rats with the 2 types of board (Figure 8 B). During the subsequent dark phase, the rats in the plain board group exhibited a significantly ($P < 0.05$) higher HR response compared with the diet board group in the IVCs and those provided with a tunnel and living in open-top cages (Figure 8 C).

**Discussion**

Virtually nothing is known about whether cage objects alter the responses of rats to routine care and research procedures (for example, cage change and gavage). Most published studies on cage change or gavage$$^{7,8,13,27,29,31-33,40}$$ either lacked cage items or details regarding the objects provided or provided the same item for all groups. Only 1 study$$^{34}$$ has addressed the effect of
The effect of cage objects on the procedures appears to include a genetic component. A previous study also showed a difference between SHR and SD rats in responses to procedures, and the authors concluded that making generalized recommendations to the animal care community regarding rat enrichment programs is difficult.

Cardiovascular telemetry allows continuous recording. The items themselves resulted in a variable baseline (Table 1), but this variability was accommodated through adding item-specific individual baseline values as covariates for both day and night responses. Consequently, the statistically significant differences obtained can be considered as true differences between groups.

Overall, even small differences may be important because these procedures are done in many animals.

Figure 7. MAP response (change ± SEM) of rats to gavage in IVCs and open-top cages. (A) First hour after cage change. (B) From 1 h after cage change to start of dark period. (C) First dark period after cage change. The y axis scale is the same in all figures. Overall there are 16 experimental units, 4 animals in 4 rounds. *, P < 0.05; #, P < 0.01; §, P < 0.001.

Figure 8. HR response (change ± SEM) of rats to gavage in IVCs and open-top cages. (A) First hour after cage change. (B) From 1 h after cage change to start of dark period. (C) First dark period after cage change. The y axis scale is the same in all figures. Overall there are 16 experimental units, 4 animals in 4 rounds. *, P < 0.05; #, P < 0.01; §, P < 0.001.

The current study demonstrates that the cardiovascular response to both cage change and gavage in F344 and BN rats is modified by the cage item added and the strain of rat evaluated. These intraspecies differences in cardiovascular responses are not surprising, given that these rat strains differ extensively in their baseline systolic and diastolic blood pressures and HR, plasma corticosterone, and brain and pituitary mineralocorticoid receptor levels. These strains also differ in their motor activity level, diurnal rhythm and behavior. Therefore, the effect of cage objects on the procedures appears to include a genetic component. A previous study also showed a difference between SHR and SD rats in responses to procedures, and the authors concluded that making generalized recommendations to the animal care community regarding rat enrichment programs is difficult.

Cardiovascular telemetry allows continuous recording. The items themselves resulted in a variable baseline (Table 1), but this variability was accommodated through adding item-specific individual baseline values as covariates for both day and night responses. Consequently, the statistically significant differences obtained can be considered as true differences between groups. Overall, even small differences may be important because these procedures are done in many animals.
The current study assessed responses to cage change during 3 subsequent time windows. The response during the first hour after the procedure is considered to result from the combined effects of lifting and transferring the rat to a new cage and its exposure to the new environment. The period between the first hour after a procedure until the start of the dark phase is considered to reflect the rat’s reaction to the new environment during the inactive (that is, lights-on) period. Finally, the subsequent 12-h active period should reveal any long-lasting consequences of the cage items.

The immediate MAP and HR responses to gavage appear to be smaller in magnitude than those associated with cage change (Figure 4 A and 7 A). In 1 study, the immediate responses in blood pressure and HR to cage change and gavage in an outbred Wistar stock were essentially the same as ours. Another study found a larger increase in the corticosterone level when rats were moved to a novel environment compared with that associated with short-term handling. Gavage is a short-term procedure that is usually considered more invasive than handling. A feature common to both gavage and handling is that the rats are returned back to the familiar home cage. In the cage change procedure, the animals are relocated to a new environment with new odors; therefore the more intense response to cage change is not surprising.

The locomotor activity of the rats increased immediately after placement into the clean cages (Figure 3). This finding agrees with several studies in which cage change has been shown to increase the motor activity of rats. Clean cages also change the behaviors displayed by rats: grooming, eating, drinking, resting, rearing, and bedding manipulation all decrease, whereas the walking and skirmishing increase immediately after cage change. As a result of the increased exploratory behavior, cardiovascular parameters and activity increase after cage changes. Cage objects can provide an odor cue that makes the new cage more familiar to rats.

The cage change procedure increased MAP, HR, and locomotor activity in all groups, but the values returned to near baseline within 1 h (Figures 3 to 5). These findings are consistent with other studies that also found increased blood pressure and HR after cage changes. However, in the cited studies, both parameters returned to baseline within 60 to 180 min. Seemingly minor differences in the cage changing procedure can have cardiovascular effects in rats. The HR of the rats is increased even by moving the cage to a different location in the cage rack. In another study, if cage changes took place in the morning, during the resting period, the systolic and diastolic blood pressures and HR responses were larger than those when cage changes occurred during the active period in the evening. Another study reported that if rats experienced a cage change during the light period, they slept less and had more chromodacryorrhea, reduced thymus weight, increased aggression, and less object-directed behavior. Consequently, the authors suggested that performing husbandry procedures during the dark period rather than the light period might improve the wellbeing of rats. This timing may be impractical, however. In our current study, cages were changed at about noon—clearly within working hours.

We used strain-specific reference values (that is, night–day differences in MAP and HR calculated for the control group) to assess whether statistically significant differences detected in the current study have biologic or welfare relevance. Based on this comparison, the statistically significant MAP responses to both cage change and gavage for F344 rats in IVCs were not greater than the night–day MAP difference for this strain (that is, 6.4 mm Hg) or the corresponding HR value [that is, 55.5 beats per minute (bpm)]. Therefore, a biologically valid effect attributable to cage objects is not apparent in the current study.

The presence of a plain board in IVC-housing BN rats influenced the MAP response to gavage until dark (Figure 7 A and B). This significant difference exceeded the BN-specific MAP night–day difference (1.8 mm Hg). However, the corresponding HR differences (Figure 8 A through C) were less than the HR night–day difference (31.4 bpm).

The MAP response to cage change of BN rats in the tunnel group, as compared with both control and diet board groups, was greater during the second sampling window (Figure 4 B), and the magnitude of the difference exceeded the corresponding night–day difference. This effect appeared to be related to the light phase, because during the subsequent dark period, the presence of the plain dividing board did not change the MAP as compared with the controls (Figure 4 C). The between-groups HR differences were not close to the corresponding night-day difference. BN rats generally rested on top of rather than inside of the tunnel and tend to be aggressive toward their cagemates. A single tunnel may not have provided sufficient numbers of compartments to function as safe havens compared with those created by inclusion of the plain board. This explanation could account for the MAP changes that occurred when a tunnel was present in the cage.

In open-top cages, the F344 night-day difference of the control group was 7.9 mm Hg for MAP and 68.0 bpm for HR. Although many statistically significant differences were detected in the responses to both procedures (Figures 4, 5, 7, and 8), none of them were large enough to achieve biologic significance. Compared with the F344 strain, BN rats in open-top cages showed lower night–day differences than they had shown 8 wk earlier when they were in IVCs (Table 1). In BN rats, the presence of the tunnel changed responses to gavage during the first hour in terms of the MAP response, as compared with the diet board and control groups (Figure 7 A), although with respect to HR, the tunnel-associated difference was significant only in comparison to the diet board (Figure 8 A). Thereafter the presence of the plain board lessened the response, especially in MAP during the subsequent dark period (Figure 7 B, C). A similar trend occurred for HR, but a biologically meaningful effect occurred only for comparison of the plain board and tunnel during the 5 h before the dark period (Figure 8 B).

After the cage change, the BN rats showed no significant differences in HR. The change in MAP in BN rats supplied with a diet board, as compared with the controls, reduced the response during the period from 1 h after cage change until dark (Figure 4 B). Perhaps the new diet board allowed easier access to food than did the old, worn-out board, thereby leading to the changes in MAP.

When living in open-top cages, rats can smell other rats in the room. This situation may influence postprocedural responses. Comparison of the cage types used in the present study is difficult due to the 8-wk age difference in the rats in the 2 cage types and the different physical environments.

Overall, cardiovascular telemetry allowed us to assess the impact of cage objects on responses to procedures. However, the importance of statistically significant effects must be interpreted with regard to biologic significance. In this regard, we detected...
no meaningful effect of cage objects on the responses of F344 rats to either cage change or gavage. In BN rats, the presence of the plain board in the IVC modified the responses to both procedures. The only effect observed in open-top cages during the first 60 min was associated with gavage and the presence of the tunnel in BN rats. In conclusion, the response of rats to various husbandry procedures appears to be specific to strain and cage objects, and perhaps also to age and cage type, complicating the establishment of valid general recommendations.

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References
CHAPTER V

Impact of aspen furniture and restricted feeding on activity, blood pressure, heart rate, and faecal corticosterone and immunoglobulin A excretion in rats (*Rattus norvegicus*) housed in individually ventilated cages.


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Impact of aspen furniture and restricted feeding on activity, blood pressure, heart rate and faecal corticosterone and immunoglobulin A excretion in rats (Rattus norvegicus) housed in individually ventilated cages

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Abstract
This study aims to evaluate the impact of adding different items in individually ventilated rat cages on the animal’s activity, cardiovascular parameters and faecal stress indicators. The following three cage items made of aspen were compared: a cross made of two intersecting boards, a similar cross where drilled holes were loaded with food pellets (restricted feeding) and a rectangular tube. Male rats of the strains BN and F344 (n = 12) were housed in groups of three; one rat in each group was implanted with a telemetric transponder to measure mean arterial pressure (MAP) and heart rate (HR). In a crossover design, each group spent 14 days with each type of cage furniture, thereafter faecal pellets were collected for faecal analyses. The means of activity and means and coefficient of variation for MAP and HR were calculated for days 2, 6, 10 and 14. As a way of determining which of the statistically significant MAP and HR mean changes were biologically meaningful, the night–day differences of the controls on day 14 were used. Both board types lowered MAP of F344 rats; hence dividing walls seem beneficial for F344 welfare. None of the MAP or HR differences in BN rats were biologically significant. No statistically significant differences in faecal corticosterone or IgA excretion were detected. In conclusion, provision of general recommendations with respect to cage furniture for rat cages is complicated because there is a clear genetic component involved in how animals respond to these structures.

Keywords: Refinement, reduction, rodents, environmental enrichment, telemetry

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Compliance with the new European regulations mandates the use of nesting material or nest boxes in rodent cages.¹,² These regulations are based on clear scientific, primarily ethological, rationale.³⁴ Regulatory compliance is easily achieved by adding structures into the cage, but not all of these may function as housing refinements, some may have no value and some may even have a negative refinement or reduction impact.⁵–⁹ The potential value of structures to be placed into rodent cages requires systematic studies to assess and compare the value of any new item or combination of items.

Regulatory texts have a tendency to generalize research findings. Indeed, the current European guidelines on laboratory animal housing and care are identical for all rodent species; and within a species they have to be implemented irrespective of animal strain, and caging system in use.¹,² One widely used type of housing is the individually ventilated caging (IVC) system, which has been shown to change the physical environment inside the cages compared with that of open cages in terms of illumination, acoustic environment, temperature and relative humidity (RH).¹⁰

When animals are stressed, their attempts to cope with their situation may be evaluated by the activity of the sympathetic nervous system, the hypothalamic–pituitary–adrenal (HPA) axis activity and their behaviour.¹¹ Common feature of all stress assessment methods is the requirement to sample by using non-disturbing and
non-invasive methods. Moreover, single indicators may be misleading, and an evaluation of improvements in cage environment often requires an assessment of a combination of indicators.\textsuperscript{12,13}

Radio telemetry is a method for measuring physiological parameters, such as blood pressure and heart rate (HR), in laboratory rodents which allows the animals to move freely during recording, making any restraint unnecessary. Indeed, the values of HR and blood pressure are considerably lower in animals implanted with a radio telemetry transmitter than the corresponding values obtained with other methods.\textsuperscript{14} The effect of the different combinations of added cage items in rats is an active study area, but telemetry assessments have rarely been incorporated into these experiments. The study of Sharp et al.\textsuperscript{15} using Sprague-Dawley (SD) and spontaneously hypertensive (SH) male rats showed that their multifaceted enrichment programme reduced HR in both dark and light periods and increased activity during the afternoons for SH rats.

Corticosteroids and their metabolites are excreted into urine and faeces, both of which can be regarded as time-integrated indices of stress. Unlike serum sampling, samples of both urine and faeces can be obtained non-invasively though the changes in the concentrations in the circulation occur with a delay of approximately 10 h in urine and 16 h in faeces.\textsuperscript{16} About 20% of the recovered metabolites in rats appear in urine and 80% in faeces.\textsuperscript{16,17}

In a similar manner to serum corticosterone,\textsuperscript{18} concentrations of its metabolites in faeces follow a diurnal rhythm; but conversely to the serum corticosterone, the highest concentrations of the faecal corticosterone metabolites appear to be present in the morning samples.\textsuperscript{16,19} If stress persists for a longer time, it may also cause immunosuppression. This can be assessed by quantification of secretory immunoglobulin A (IgA) in faeces. Faecal IgA excretion also exhibits a diurnal variation, but the values detected in morning and evening samples have been somewhat controversial.\textsuperscript{19–21} Faecal samples for both assays are easy to collect from the cage without disturbing the animals, enabling long-term, longitudinal studies.\textsuperscript{22}

Rats are crepuscular and nocturnal animals and their blood pressure and HR are higher during the night than during the day, but there are major differences between different stocks and strains.\textsuperscript{15,23–26} In the present study, two rat strains, F344 and BN, were studied in order to elucidate whether there would be any genetic contribution to the variation in the results, and to achieve better applicability within the species.\textsuperscript{27} These strains were chosen because they differ in various aspects of their physiology, including locomotor activity level, diurnal rhythm of activity,\textsuperscript{25,26} blood pressure, HR\textsuperscript{25} and levels of circulating corticosterone.\textsuperscript{29,30}

Laboratory rodents are commonly fed \textit{ad libitum}, e.g. food is available all the time, a feeding regime known to cause obesity, increase the incidence of diseases and shorten lifespan in rats.\textsuperscript{31–33} On the other hand, restrictive feeding may be difficult to combine with the recommended group housing of rats,\textsuperscript{1,2} as there is no practical or effective way to evenly restrict the food intake of all individuals within the group. Rats eat predominantly during the dark period when the food is available \textit{ad libitum}\textsuperscript{35–36} and when the restricted feeding is timed in the light phase in rats, it may impair both natural feeding patterns and gastrointestinal physiology. The diet board, where the access to food requires rats to gnaw wood, has been shown to significantly reduce the weight gain in rats with 15–18% less food being eaten.\textsuperscript{28}

We hypothesized that a refinement value could be detected via changes in mean arterial pressure (MAP), HR, faecal corticosteroid metabolites and faecal IgA, and a reduction value by changes in the coefficients of variation (CV) of these parameters, this being attributable to various cage items and restricted feeding. This study was designed to evaluate the impact of dividing aspen walls with or without restricted feeding, as well as an aspen tube on laboratory rats using activity, circulatory parameters and faecal stress indicators to determine whether two different rat strains would display variation in their responses, and whether there would be habituation to the items.

\section*{Materials and methods}

The study was carried out in the Laboratory Animal Centre, University of Helsinki. The protocol of the study was reviewed and approved by the Animal Ethics Committee of the University of Helsinki.

\section*{Animals}

A total of 12 BN (BN/RijHsd) and 12 Fischer344 (F344/NHsd) male rats (Harlan, Horst, The Netherlands) were used in this study. The rats were 25 weeks old and weighed 280–370 g (BN) or 350–460 g (F344) at the beginning of the study. Upon arrival from the commercial breeder at the age of 10 weeks, the animals were housed in IVCs with the same bedding material and the same social groups as during the study.

\section*{Animal housing and care}

All rats were housed in the same room in groups of three rats per cage in polysulphone IVCs (Tecniplast, Buguggiate, Italy). The cage type used was 1500U Eurostandard IV S (44.5 × 33.5 × 21.0 cm – floor area 1500 cm\textsuperscript{2}) with a solid bottom and IVC-double lids. The cage floor was covered with 3.0 L aspen chip bedding (\textit{size} 4 × 4 × 1 mm, 4HP, Tapvei Oy, Kaavi, Finland). The cages were changed once weekly. The room temperature was 21.2 ± 0.3 °C (mean ± SD) and the RH was 53.5 ± 7.7%, but actual values inside the IVCs were 1–4 °C and up to 6% RH higher. Artificial lighting with fluorescent tubes (light colour warm white) was on from 06:00 to 18:00 and the light intensity in cages 1 m above the floor was 6–9 lx. The sound level with R-weighting (adjusted for the hearing sensitivity of rats), in empty IVC cages, was 20–25 dB(R), with the corresponding A-weighting (adjusted for the hearing sensitivity of humans) being 45–47 dB(A).\textsuperscript{37} Tap water was provided in polycarbonate bottles, changed once a week and refilled once in between. Feeding was restricted or \textit{ad libitum} depending on treatment as outlined below. For a more thorough description, see Kemppinen et al.\textsuperscript{10}
Cage furniture and study design

Animals were housed in permanent groups of three. The experiment utilized a crossover design with two-week periods and a rotational order between control and the following three cage furniture items (Figure 1):

1. Cage without furniture (control);
2. Cage equipped with a cross made of intersecting two aspen boards (34.0 × 14.7 × 3.2 and 21.1 × 14.7 × 3.2 cm, plain board);
3. The same as (2) but holes (12 mm) were drilled into the boards and then were loaded snugly with food pellets; rats had to gnaw wood to gain access to the food, no other food source was available (diet board);
4. Cage provided with a rectangular aspen tube (20.0 × 12.0 × 12.0 cm, external dimensions).

The order of the cage items was arranged at random, and the first item in each group was randomly allocated. The illustration of the item order can be seen elsewhere.

The items were made of aspen because this was the same material as the bedding and they can be assumed to have the same volatile compound emissions, in this case especially the absence of α- and β-pinenes, but also to be able to endure several bouts of sanitation. The day when the rats were introduced to the new cage furniture was designated day 1 in all study periods. Rats were changed to clean cages on day 8 at noon during every period.

Irradiated (25 kGy) pelleted feed (2016 Global Rodent Maintenance, Harlan Teklad, Bicester, UK) was available to the three groups (control, plain board, tube) ad libitum, while the diet board group had the food pellets in holes drilled into the aspen board. When fed with the diet board, rats have been shown to eat 12–18% less and gain weight slower.

Surgical procedure

Eight rats were implanted with a radiotelemetry transmitter (TA11PA-C40; Data Sciences International, St Paul, MN, USA). Anaesthesia was induced with a mixture of fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium; one part), midazolam (Dormicum®, Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany; one part and two parts of sterile water) at the doses of 0.15–0.20 mL/100 g subcutaneously. The ventral abdomen area was shaved and scrubbed with MediScrub®, 1% triclosan solution (Medichem International, Sevenoaks, UK) and disinfected with chlorhexidine solution (Klorohexol® 5 mg/mL, Leiras, Turku, Finland). Ocular lubricant (Viscotears®, Novartis Healthcare, Copenhagen, Denmark) was applied to both eyes. After a small incision on the ventral abdominal midline had been made, the presoaked transmitter was placed into the abdominal cavity and the catheter into the abdominal aorta. The transmitter was secured into the abdominal wall with 4–0 Ethilon® II sutures (Johnson & Johnson Intl, St Stevens-Woluwe, Belgium) and the incision was closed with 5–0 Ethicon® Vicryl® (Johnson & Johnson Intl). The surgery procedure lasted about 20–30 min. Postoperative pain alleviation was carried out with 0.01–0.05 mg/kg subcutaneous buprenorphine (Temgesic®; Schering-Plough Europe, Brussels, Belgium) twice a day and once a day a dose of 5 mg/kg subcutaneous carprofen (Rimadyl®; Vericore Ltd, Dundee, UK) for at least three days, and parenteral fluids were given for three days. On these three days, the implanted rats were housed alone and then placed back into their home cages. The pain medication for each rat was titrated against the individual response. The animals were allowed to recover for 10 days before the experiment was started.

Sampling

Mean blood pressure, HR and locomotor activities were recorded telemetrically every 75 s for 24 h on days 2, 6, 10 and 14. In order to conduct the locomotor activity measurements, the telemetric receiver had two antennae, located at two sides, like an x- and y-axis, and the receiver detected the difference in signal strength when the animal moved in relation to these antennae.

At the end of each period, the rats were housed singly for 6 h (06:00–12:00) and all faecal pellets voided from each
individual were collected and frozen (−18°C). All samples were analysed individually.

Faecal corticosterone and faecal IgA quantification

The extraction of both corticosterone and IgA was performed as described by Pihl and Hau19. The corticosterone enzyme-linked immunosorbent assay was performed using a commercial corticosterone kit (DRG Diagnostics, Marburg, Germany) using the manufacturer’s instruction manual. The quantification of IgA was performed using the assay also described by Pihl and Hau19 and reagents were obtained from AbD Serotec (Kidlington, Oxfordshire, UK; purified rat IgA standard, PRP01), concentrations 0–1000 ng/mL; coating antibody (mouse anti rat IgA heavy chain, MCA191); and detection antibody (mouse anti rat kappa/lambda light chain: HRP, MCA1296P, diluted 1:1000).

Data processing and statistical analysis

The number of animals needed in the study was estimated with the resource equation method.22 The cage was used as an experimental unit, and with the crossover design used, this resulted in 12 degrees of freedom for error per strain, well within the optimum range, i.e. 10–20. Means and CV for MAP, HR and mean for locomotor activity were calculated in the light and dark periods for days 2, 6, 10 and 14 in each period for 30 min intervals from the raw data. The 12 h mean values for MAP and HR of the controls were subtracted from those of plain board, diet board and tube groups for the same days, and CV values of four furniture items were used as such. Mixed-model repeated measures analysis of variance (ANOVA) on SPSS for Windows (version 14.0) was used, followed by Bonferroni correction for post hoc comparisons. For locomotor activity, furniture item was used as the main effect and age as covariate. For MAP, HR and all CVs, furniture item was used as the main effect and age and activity as covariates. Analogous calculations were carried out to compare data within a furniture item between the days; the day was used as the main effect instead of furniture item. We assumed that for a cardiovascular effect of housing to be biologically meaningful, it would have to be at least as large as the difference between night and day values. Therefore, mean night–day difference was calculated from day 14 data for the control group of both rat strains.

Significant CV differences between groups were processed further to point estimates [−(CV1/CV2)²]. Significance was set at P < 0.05 for mean value calculations, but at P < 0.01 for CV statistics to increase the reliability of the findings.

The faecal corticosteroids and faecal IgA results of three rats from the same cage were averaged and calculated with repeated measures mixed-model ANOVA using the age as a covariate.

Results

F344 rats were far more active than the BN rats during the dark phase, whereas the activity during the light phase was similar in these strains (Figure 2). In the dark phase, F344 rats were significantly (P<0.01) more active in cages with diet boards than in the control cage on day 6, while on day 10, they exhibited significantly (P<0.05) higher activity with the tube compared with those living with the diet board and controls (Figure 2a), but these differences disappeared by the end of the two-week period. The BN rats exhibited no differences in activity between the cage furniture items (Figure 2b).

When calculated from data of both strains and all the groups, F344 rats displayed higher values of MAP and HR than BN rats throughout the study. In both dark and light phases, MAP exhibited a significant (P<0.001) group × strain interaction on all observation days. HR showed a significant (P<0.05) group × strain interaction during the dark phase from day 6 onwards. Due to the multiple interactions encountered, the following results will be presented separately for both strains and for each light phase.

Effect of housing item

F344 rats

MAP and HR night–day differences for F344 controls were 5 (±2) mmHg and 52 (±13) BPM at day 14. Throughout the two-week periods, both during the dark and light phases, F344 rats living with the tube exhibited increased MAP, while the other two items decreased MAP compared with the baseline of the controls; all these comparisons being statistically significant (P<0.001). When housed with the plain board, rats had lower MAP on day 6 light phase (P<0.001), on day 10, dark phase (P<0.01) and on day 14 all throughout the 24 h (P<0.01–0.05). In most cases, these differences were also biologically significant, i.e.
exceeded 5 mmHg (Figure 3a). HR changes are shown in Figure 3b with statistical significances, but none of the differences came even close to the HR night–day difference of the F344 control group, i.e. 52 BPM.

In F344 rats on day 2, the CV of MAP was significantly higher when the animals were housed with the plain board than with the two other items and the control cages during both the dark (all \( P < 0.01 \)) and the light (all \( P < 0.001 \)) phases. On day 10 in the light, the CV of HR was significantly lower in the diet board animals than in the control cages (\( P < 0.01 \)). On day 14, the CV of MAP in the light phase was significantly (\( P < 0.01 \)) higher with the plain board compared with the tube (\( P < 0.01 \)), while CV of HR in the dark phase was significantly lower with the plain board cages than in the tube cages (\( P < 0.01 \)). All these results are shown graphically in Figures 4a and b and in table format with the corresponding point estimates in Table 1.

**BN rats**

The night–day differences in MAP and HR for BN controls were 3 (±1) mmHg and 25 (±7) BPM at day 14. In BN rats, there were few statistically significant changes in MAP and HR between the different cage items. Overall MAP was significantly (\( P < 0.001–0.05 \)) lower in the diet board cages than in the plain board cages early in the period, this became less and finally it stabilized as the lowest of all the groups at the end of the two-week period (Figure 3c). Nonetheless, it was only by day 14 that the MAP decrement of the diet board, as compared with the plain board, exceeded the limiting night–day difference. The relationship of the HR between the cage furniture items was rather similar (Figure 3d), but in analogy to F344 rats, none of the differences reached a biologically significant value. In BN rats on day 14 in the light phase, the CVs of the MAP and HR were significantly lower in the diet board cages than in the control cages (\( P < 0.01 \)). All possible comparisons are illustrated graphically in Figures 4c and d and those statistically significant in table format with the corresponding point estimates in Table 1.

**Between days comparison**

When the days were compared within the furniture item during the two-week period in both strains, there was a decreasing trend both in MAP and HR compared with the control baseline, and although between-day differences were small, they were in most cases statistically significant (\( P < 0.001–0.05 \)), particularly in the F344 rats (Figure 5).

**Faecal assays**

There was a large variation in the number of faecal pellets voided, from none up to more than 10 pellets from one animal. No group \( \times \) strain interaction was seen, and none of the two strains exhibited any between-furniture item differences in the faecal corticosterone or in faecal IgA (results only in F344 rats) excreted (Figure 6).

**Discussion**

The F344 rats consistently had higher MAP and HR than BN rats, and the dark activity levels of the F344 rats were also...
higher than those of BN rats (Figure 2). This is well in line with the results of van den Brant et al. who concluded that the BN strain no longer possesses the typical rodent nocturnal activity. The present study showed that in comparable environments, F344 rats display larger night–day differences both in MAP and HR values compared with the BN rats. This agrees with the results of van den Brant et al. who categorized the F344 as a ‘hypertensive’ and the BN as a ‘hypotensive’ rat strain. We compared all the MAP and HR level differences to the corresponding night–day difference of the controls, and propose that statistically significant differences in means should exceed the normal night–day difference, in order to be considered to be biologically meaningful. The results of this study suggest that a fixed percentage, e.g. 6–7% as suggested by Krohn et al. may not be applicable for all strains, and that a strain-specific measure, such as night–day difference, may be more valid for this purpose.

This study shows that cardiovascular responses of F344 rats and BN rats to the added furniture items differed. The F344 rats had the highest MAP in the tube cages all the time throughout the two-week period (Figure 3a). It has been shown that albino Wistar rats prefer a cage with a shelter rather than a barren environment and that they tend to stay inside the tube during the light phase. However, it is possible that the tubes used in the present study may have been too small to comfortably accommodate three large rats, and that the finding is applicable to the F344 strain only.

Table 1 P values for significant mean arterial pressure (MAP) and heart rate (HR) coefficient of variation (CV) comparisons between the groups and corresponding point estimates (PE) for both F344 and BN rats and both light phases on the observation days.

<table>
<thead>
<tr>
<th></th>
<th>F344 rats, P &lt; PE</th>
<th>BN rats, P &lt; PE</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MAP dark HR dark</td>
<td>MAP light HR light</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain board/diet board</td>
<td>0.01/1.36</td>
<td>0.001/1.42</td>
</tr>
<tr>
<td>Plain board/tube</td>
<td>0.01/1.56</td>
<td>0.001/1.61</td>
</tr>
<tr>
<td>Plain board/control</td>
<td>0.01/1.41</td>
<td>0.001/1.52</td>
</tr>
<tr>
<td>Day 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control/diet board</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain board/tube</td>
<td>0.01/0.70</td>
<td>0.01/1.45</td>
</tr>
<tr>
<td>Control/diet board</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Non-significant comparisons are not shown in the table. NS = not significant
Both board structures lowered the MAP values of F344 rats throughout the two-week period and towards the end, the periods with the plain board seemed to be most effective in this respect (Figure 3a). Based on this observation, it is likely that the dividing walls represent a suitable cage item for F344 rats, but also the diet board appears better than the tube, and certainly also better than no furniture at all. It is interesting to note that both boards, unlike the tube, could not be used to provide cover, a feature mandated by the European guidelines.

The diet board involves the concept of work for food, a feature that would be expected to raise at least the active period MAP, but nonetheless the difference was minor compared with the plain board. For routine animal observation purposes, dividing walls are clearly better than covered structures that obscure the animals. This seems to challenge the concept that all albino rats need to be provided with structures for hiding places and privacy.44,45 The BN rats exhibited no biologically significant MAP or HR between-furniture item differences.

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Only a handful of earlier studies on the effect of new cage items have utilized telemetry. Sharp et al.15 evaluated their enrichment programme in rats with telemetrically recorded HR, systolic blood pressure and activity. They showed that HR of SH rats was significantly reduced by their combination of cage items, but for the SD rats they could detect no effect. Their programme consisted of several smaller items added to the cage between three and seven days.

Figure 5 The mean arterial pressure (MAP) and heart rate (HR) differences (SEM) to controls of F344 (a and b) and BN (c and d) rats with three cage items during the dark and light periods in individually ventilated cages (IVC). BPM: beats per min; *P < 0.05; **P < 0.01; ***P < 0.001. Number of experimental units = 16

Figure 6 Corticosterone (a) and immunoglobulin A (IgA) (b) excreted (SEM) via faeces for F344 and BN rats with three cage items during the dark and light periods in individually ventilated cages (IVC). Number of experimental units within a strain = 16
There were some similarities between these items and those used in our study, such as the simulated burrow, which is comparable to the tube used in the present study, and a gnawing and food foraging item, which is comparable to the diet board of our study. A detailed comparison between the two studies is not relevant because we used one item at a time for 14 days, as opposed to their combination of items and much shorter exposure time.

When the processed days were compared within the furniture item during the two-week periods, there was a decreasing trend both in MAP and HR levels in both strains (Figure 5), but statistical and biological significant findings were only found for MAP of F344 plain board rats when comparing days 2 and 14. We interpret this to mean that the F344 rats had habituated to the plain board, but not to the other items. Conversely, no such effects were detectable in BN rats.

The CV of MAP in F344 rats was highest in the plain board groups on day 2, both in the dark and light phases. This may simply be a novelty effect of this cage item which was introduced into the cage one day before (Figure 4a). The corresponding point estimates showed that with the plain board and MAP as the result parameters, the number of animals needed would be 1.36–1.81 times greater than those with other items including controls. Similar results were found between plain board and the tube on day 14 dark (Table 1). The high MAP seen in the F344 rats living with the tube was not associated with excessive variation but rather the opposite. In the BN rats, the CV values of both MAP and HR were higher in the control groups compared with the diet board groups (Figure 6) and the corresponding point estimates were 1.45 and 1.68, respectively (Table 1). Cage furniture may thus have marked, strain specific, effects on the within-group variation and this could be anticipated to influence the number of animals needed in blood pressure studies.

In the present study, the cage furniture items used exhibited no effect on faecal corticosterone or IgA excretion and there were no differences compared with the rats housed in control cages. Sarrieau and Mormede et al. have shown significantly higher plasma corticosterone levels in F344 rats compared with BN rats, but we could not detect any significant differences in faecal corticoids between the strains (Figure 6a). A recent study by Siswanto et al. showed that injection of at least 100 µg/kg adrenocorticotropic hormone is required to detect a subsequent change in the faecal corticosteroid level in rats. Hence, it was not surprising that the HPA axis was not sufficiently stimulated by the changes in cage environment for this to be detectable as changes in faecal corticosteroid excretion. Faecal IgA concentrations in rats have also been measured in response to injection of at least of 100 µg/kg adrenocorticotropic hormone. However, there were no differences between those of corticosteroids; hence, IgA may be useful for assessing long-term animal wellbeing. Obviously, the lack of significant changes in this study suggests that two weeks may not have been long enough or alternatively the response was simply too small to be detected with this assay.

In conclusion, cardiovascular parameters can be used to assess the welfare value of cage furniture, whereas changes in faecal corticosterone and faecal IgA excretion would appear to be too small to be quantifiable. Furthermore, covered structures may not be any better than no structure at all in the cage, and the establishment of general recommendations may be difficult because there is a clear genetic component involved, resulting in major between-strain differences.

ACKNOWLEDGEMENTS

This study was supported by the Academy of Finland, the Finnish Ministry of Education, ECLAM and ESLAV Foundation, the North-Savo Cultural Foundation, the Finnish Research School for Animal Welfare, the Oskar Öflund Foundation and the University of Kuopio. We are thankful to Mr Heikki Pekonen for surgical procedures and to Ms Virpi Nousiainen for daily care of the rats.

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CHAPTER VI

Refinement and reduction value of aspen furniture and restricted feeding of rats in conventional cages.

Niina Kemppinen, Anna Meller, Jann Hau, Kari Mauranen, Tarja Kohila and Timo Nevalainen.

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Summary
This study evaluated the impact of aspen furniture on cardiovascular parameters, locomotor activity (LA) and faecal welfare indicators in rats. A total of 12 BN and 12 F344 male rats were group housed (n=3) in conventional cages. Two groups received a simple maze, one group received a rectangular tube and the controls had no furniture. In one of the two maze groups, the rats had to gnaw through wood in order to obtain food. The mean values of the LA in all groups and differences in mean arterial pressure (MAP) and heart rate of the rats housed in the various item groups were compared to the values of the rats housed in control cages with no furniture, on days two, six, ten and 14 in each period (both light and dark phases). The F344 rats were generally more active than the BN rats during the dark phase, but not during the light phase. Based on the MAP results, the tube appeared to be a poor choice for F344 rats, while for BN rats all furniture items seemed beneficial, with both board types apparently superior to the tube. In general, F344 rats had higher faecal corticosterone levels than BN rats with the reverse being true for secretory IgA values. In conclusion, LA and cardiovascular parameters seemed appropriate ways to evaluate the impact of cage furniture on physiological parameters, and covered structures such as tubes do not seem to provide any enrichment value in these two rat strains.

Introduction
Rats prefer cages containing a nest box (Patterson-Kane, 2003; Townsend, 1997) and they are willing to work in order to gain access to cages furnished with a box or nesting material (Manser et al., 1998). New European regulations state that if there is not enough nesting material available to build a complete, covered nest, a nest box should be provided (Council of Europe, 2006; European Union, 2007). Dividing structures and shelters in the cage may offer rats opportunities to seek or avoid contact with other group members and are regarded as beneficial to animal welfare (Stauffacher et al., 2002).

The benefits and problems of housing refinement - also called environmental enrichment - achieved by inclusion of different items or materials in the cage, need to be evaluated because the putative positive effect on the welfare of the animals may depend on various characteristics of the animals, e.g. strain, stock, sex, and age. Some structures may even have a negative impact on animal welfare (Kaliste et al., 2006; Moncek et al., 2004; Tsai et al., 2002; Tsai et al., 2003). A true value assessment requires systematic evaluation of the individual items and the relevant combinations of items.

A stressful environment activates the autonomic nervous system (ANS) causing persistent elevations of both heart rate (HR) and blood pressure. A lowering of HR and blood pressure may thus be considered to reflect an increase in the welfare of the animals. Radiotellemetry as a method to record physiological parameters, such as HR and blood pressure, allows animals to move freely while recording i.e. restraint is unnecessary. The values of HR and blood pressure are thus considerably lower in animals implanted with a telemetry transmitter than those obtained with other methods (Kramer et al., 2001).
Telemetry has been used to analyze the impact of different cage flooring in rats (Krohn et al., 2003a), and Sharp et al. (2005) studied a multifaceted enrichment program’s effect in Sprague-Dawley (SD) and spontaneously hypertensive (SHR) male rats. The cage enrichments had no significant effects on basal or undisturbed HR, systolic blood pressure (SBP) or activity in SD rats or on SBP in SH rats. However, in SHR rats with enrichment, the HR was reduced in both dark and light phases and the SHR rats’ activity increased during the afternoons. In our previous study, cage furniture had item- and strain-specific effects on blood pressure and HR in BN and F344 rats housed in an IVC-system, and the rats exhibited habituation to some of the items (Kemppinen et al., in press).

Increases in the serum corticosterone level are attributable to activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to stressful stimulation in rats; but this response is not as rapid as that of ANS. Corticosteroid metabolites are excreted both into urine and faeces, but unlike the situation in serum, they are not detectable until 6-10 hours later in urine and 4-1216 hours later in faeces after stressful event (Bamberg et al., 2001, Royo et al. 2004, Lepschy et al. 2006, Siswanto et al. 2008, Abelson et al. 2009). The major pathway of excretion in rats is via faeces; this accounts for 80 % of the recovered metabolites in rats (Bamberg et al., 2001; Lepschy et al., 2007). One major advantage of faecal samples for quantification of stress sensitive molecules is that faeces are voided voluntarily and there is no need to handle animals, and even if there is some response to the collection procedure, the delay before corticosteroids appear in the faecal pellets ensures that corticosteroid levels in the samples collected are not affected by events associated with the sampling procedures (Bamberg et al., 2001; Möstl & Palme, 2002).

Prolonged stress may lead to immunosuppression. The levels of secretory immunoglobulin A (IgA) in saliva have been used to assess welfare status associated with different housing conditions (Guhad & Hau, 1996). Another option is to quantify faecal IgA (Eriksson et al., 2004; Pihl & Hau, 2003; Royo et al., 2004). There are few studies in rats assessing the value of furniture items using corticoid measurements and these are often conflicting. Belz et al. (2003) showed that singly housed SD rats of both sexes housed with environmental enrichment had lower baseline plasma corticosterone levels than rats in standard cages, whereas Moncek et al.(2004) detected significantly higher corticosterone levels in Wistar male rats housed in enriched cages. However, in the latter study, multiple combinations of various items were used and the effect of any single item could not be differentiated. Nevertheless, the combination did not seem to improve the housing environment of the rats in terms of lowering corticosterone levels.

There is ample evidence that ad libitum feeding causes obesity, increases the incidence of disease, and shortens lifespan in rats (Hubert et al., 2000; Roe, 1994; Roe et al., 1995). Current legislation mandates group housing (Council of Europe, 2006; European Union, 2007), and there is presently no practical, effective way to restrict feeding when rats are group housed. When food is available ad libitum rats eat predominantly
during the dark phase (Spiteri, 1982; Strubbe & Alingh Prins, 1986; Strubbe et al., 1986). Feeding rats only during the light phase may have an impact on gastrointestinal motility and physiology. The diet board, where food is available *ad libitum*, but rats had to gnaw through wood to obtain food, resulted in 15-18% less food being consumed and a reduced body weight gain in the rats (Kemppinen et al., 2008a).

We hypothesized that a combination of cardiovascular telemetry, measurement of locomotor activity, combined with assays of faecal corticosteroid metabolites and faecal IgA, would constitute efficient tools for the assessment of refinement and reduction potential of various enrichment items for rats. This study was designed to evaluate the impact of dividing aspen walls with or without restricted feeding and an aspen tube on laboratory rats using these parameters. Additional aims were to determine whether there would be a genetic component in responses and whether the rats habituate to the items chosen for scrutiny.

**Materials and Methods**

The study took place in the Laboratory Animal Centre, University of Helsinki. The protocol of the study was reviewed and approved by the Animal Ethics Committee of the University of Helsinki.

**Animals.** A total of 12 BN (BN/RijHsd) and 12 Fischer344 (F344/NHsd) male rats (Harlan, Horst, The Netherlands), were used in this study. The rats were 33 weeks old and weighed 310 - 430 g (BN) or 380 - 480 g (F344), respectively, at the beginning of the study. Before the experiments, all rats were housed in IVCs and used in the study with the same method as in the present study (Kemppinen et al., in press).

**Animal housing and care.** Rats were housed in the same-strain groups of three in solid bottom polysulfone cages (Tecniplast, Buguggiate, Italy, ***Eurostandard IV S, 44.5 x 33.5 x 21.0 cm*) with a stainless steel wire mesh lid. The cage floor was covered with 3 l aspen chip bedding (size of 4 x 4 x 1 mm, 4HP, Tapvei Oy, Kaavi, Finland). The rats were moved to clean cages on day eight at noon in every two-week-period. Tap water was provided in polycarbonate bottles which were changed once a week at the cage change and refilled once in between.

The room temperature was 21.2 ± 0.3°C (mean ± SD) and the relative humidity (RH) 53 ± 6%. Lighting with fluorescent tubes was on from 06.00 to 18.00 with light intensity in cages 1 m above floor of 16-18 lx. The sound level, adjusted with R-weighting for the hearing sensitivity of rats, in empty cages was 12-18 dB(R), with the corresponding adjusted A-weighting - adjusted for human hearing sensitivity - being 46-49 dB(A) (Björk et al., 2000). For a more thorough description, see Kemppinen et al (2008b).

**Cage furniture and study design.** The experiment utilized a crossover design with two week periods and a rotational order. Within both strains there were two different kinds of dividers made of intersecting two aspen boards (34.0 x 14.7 x 3.2 cm; 21.1 x 14.7 x 3.2 cm), a rectangular aspen tube (20.0 x 12.0 x 12.0 cm, external dimensions), or controls without any furniture. One divider type included drilled holes for food pellets, where the rats had to gnaw for food (diet
board); the other type was without drilled holes and food pellets (plain board). The items were made of aspen as was the bedding; both have the same volatile compound emissions, in this case especially the absence of α- and β-pinenes (Nevalainen & Vartiainen, 1996), and aspen furniture endures several bouts of sanitation (Voipio et al. 2008). The day when the rats were moved between cages differing with respect to furniture item was designated as day one in every study period. Diet boards had to be renewed on day eight in order to ensure that food was always available. The order of the cage items was set at random, and the first item in each group was randomly allocated. The illustration of the items and item order can be seen elsewhere (Kemppinen et al., in press; Kemppinen et al., 2009).

Irradiated (25 kGy) pelleted feed (2016 Global Rodent Maintenance, Harlan Teklad, Bicester, UK) was provided to three groups (plain board, tube and control groups) ad libitum, while the diet board group had the food pellets embedded snugly in drilled holes (diameter 12 mm) of the aspen board.

Surgical procedure. Eight rats (one rat in each group) were implanted with a radio telemetry transmitter (model TA11PA-C40; Data Sciences International, St.Paul, MN, USA). The cylinder shape transmitter body (3.0 cm, diameter 1.5 cm) monitored pressure and activity via a fluid filled catheter (8.0 cm long) sending the signals to an electronics module. The electronics module translated the signals into a digitized form and transmitted them to the receiver plate located under the cage.

The rats were anesthetized with the combination of fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium) + midazolam (Dormicum®, Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany, 0.15 - 0.20 ml/100g SC). The abdominal area was clipped and then scrubbed with MediScrub®, 1 % triclosan solution (Medichem International, Sevenoaks, UK) solution and disinfected with chlorhexidine solution (Klorohexol® 5 mg/ml, Leiras, Turku, Finland), and an ocular lubricant (Viscotears®, Novartis Healthcare, Copenhagen, Denmark) was applied to both corneas. A sterile drape was placed over the surgical area and a small area cut away to enable a 3 cm incision to be made through the skin along the abdominal midline. The sterile transmitter was pre-soaked in sterile saline for at least 20 min before the surgery and then placed into the abdominal cavity, and the catheter into the abdominal aorta. The transmitter was sutured into the abdominal wall with 4-0 Ethicon® Ethilon®II (Johnson & Johnson Intl, St-Stevens-Woluwe, Belgium) and the abdominal and skin incisions were closed with 5-0 Ethicon® Vicryl® (Johnson & Johnson Intl, St-Stevens-Woluwe, Belgium). The surgery procedure lasted about 20-30 minutes.

After the surgery, the animals were given, twice a day, 0.01 – 0.05 mg/kg SC buprenorphine (Temgesic®; Schering-Plough Europe, Brussels, Belgium) and once a day a dose of 5 mg/kg SC carprofen (Rimadyl®, Vericore Ltd., Dundee, UK) and parenteral fluids for three days. The pain medication for each rat was titrated according to individual response. All rats were given initially buprenorphine at the highest dose; this was continued for at least two days; and carprofen medication for at least three days. On these
three days the implanted rats were housed alone and then placed back into their home cages. The animals were allowed to recover for ten days before the experiment was started.

**Sampling.** Values of mean blood pressure (MAP), heart rate (HR) and locomotor activity (LA) were transmitted every 75 seconds to the computer throughout the study. For LA measurements the telemetric receiver had two perpendicular antennas, and the receiver detected signal strength change when the rat moved in relation to these antennas.

At the end of each period, the rats were housed singly for six hours (06.00-12.00) and all faecal pellets voided from each individual were collected and frozen (-18°C).

**Faecal corticosterone quantification.** The extraction of both corticosterone and IgA was performed as described by Pihl and Hau (2003). The corticosterone ELISA was performed using a commercial corticosterone kit (DRG Diagnostics, Marburg, Germany) using the manufacturer’s instruction manual. The quantification of IgA was performed using the assay described by Pihl and Hau (2003) and reagents were obtained from AbD Serotec, (Kidlington, Oxfordshire, UK; (purified rat IgA standard, PRP01, concentrations 0-1000 ng/ml); coating antibody (mouse anti rat IgA heavy chain, MCA191); and detection antibody (mouse anti rat kappa/lambda light chain: HRP, MCA1296P) diluted 1:1000).

**Data processing and statistical analysis.** The number of animals needed in the study was estimated with the resource equation method (Festing, 2002). The cage was used as an experimental unit, and with the crossover design used, this resulted in 12 degrees of freedom for error per strain, well within the optimum range, i.e. 10-20. Means and coefficient of variation (CV) for MAP and HR were calculated at 30 min intervals for light and dark phases on days two, six, ten and 14 of each period. The mean values in all groups and differences in MAP and HR of the three furniture groups as compared to the control were calculated for these days; LA values were used as such. Mixed-model repeated measures ANOVA (SPSS Windows, version 14.0, SPSS Inc., Chicago, IL, USA) was used combined with Bonferroni correction. For LA, group was used as main effect and age as a covariate. For means and CV of both MAP and HR, LA was used as second covariates. Significant CV differences between groups were processed further to point estimates \(\left[\frac{CV_1}{CV_2}\right]\). Significance for mean comparisons was set at \(p < 0.05\), and for increased certainty at \(p < 0.01\) for CV comparisons. In order to determine which of the statistically significant cardiovascular mean changes were biologically meaningful, i.e. have welfare value; the mean night-day difference of day 14 was calculated for the control group of both rat strains.

The faecal corticosterone and faecal IgA results of each rat from the same cage were averaged at the cage level and calculated with repeated measures mixed-model ANOVA with Bonferroni correction, using age as a covariate.

**Results**

F344 rats were more active than the BN rats during the dark phase, whereas LA during the light phase was similar in these strains (Figure 1). The F344 rats, on day ten and in
the dark, were significantly (P < 0.001-0.05) more active with the tube compared to the two boards, and in the control group compared to the plain board group.

F344 rats had significantly (P < 0.001) higher MAP and HR than BN rats throughout the study. MAP exhibited a significant (P < 0.001-0.05) group*strain interaction in the dark on days six, ten and 14, and in the light on days two, ten and 14. HR showed significant (P < 0.001-0.05) group*strain interaction in dark and light phases throughout the study, except in the dark on day ten. Because of the multiple interactions encountered, the following results will be presented separately for both strains and for each lighting phase. The MAP and HR daily 30 min means of the control group were subtracted from those of the rats with the different cage items. Hence values above the control baseline designate an elevation of the parameter, and values below the control baseline represent the opposite.

**F344 rats.** The night-day difference on day 14 in the F344 control group was 10 (±3) mmHg for MAP and 60 (±29) beats per minute (BPM) for HR. On day two in the dark phase, the F344 rats in the diet board cage showed significantly (P < 0.001) lower MAP than the rats in the tube and plain board groups, and on day 14 the same was true in comparison to the tube group. On day 14, in the light phase, the rats in the tube cages had significantly (P < 0.001) higher HR than rats housed with the two other items (Figure 2a). However, none of these MAP differences in F344 rats reached biological significance i.e. exceeded day 14 night-day difference.

The HR of the F344 rats was significantly (P < 0.05) higher in the diet board cage compared to the tube cage on day two in the light phase. On day 14, both lighting phases exhibited the highest HR with the tube; with lights on the HR was significantly higher in the tube (P < 0.01) compared to the plain board; in the dark compared to the diet board (P < 0.001, Figure 2b). Similarly to MAP, no HR comparison of F344 was close to biological significance.

The F344 rats showed no differences in MAP coefficient of variation (CV), and only one significant HR CV value was observed, *i.e.* between the tube and controls during the light phase of day 14 (P < 0.01). These results are shown graphically in Figure 3 and corresponding point estimates in Table 1.

**BN rats.** In the BN rats, the day 14 night-day difference for the control group was 3 (±3) mmHg for MAP and 21 (±4) BPM for HR. Between item comparisons showed significant MAP differences only on day 14; the tube was significantly (P < 0.05) less effective in lowering MAP than the other two items. These statistical significances are unlikely to be of biological significance, since they are about 3 mmHg, similar to the night-day difference value for day 14. Although all furniture groups' MAP appeared to be below the control values, only on day 14 did both board groups consistently achieve significance (P < 0.05 - 0.01); and but this lowering effect compared to tube didn’t exceed 3 mmHg (Figure 2c). On day two, in the light phase, rats in the tube group had significantly (P < 0.05) higher HR than the diet board rats. Additionally, also in the light phase, both on days six and 14, the HR was
Figure 1. Locomotor activity (SEM) of F344 (A) and BN (B) rats with different cage items and controls during the dark and light phases. Abbreviations: * = P < 0.05, ** = P < 0.001. Number of experimental units within a strain = 16.
A. MAP difference (±SEM) - F344 rats

B. HR difference (±SEM) - F344 rats
Figure 2. The MAP and HR differences (SEM) of F344 (A and B) and BN (C and D) rats to controls with three cage items during the dark and light phases. Abbreviations: MAP=Mean Arterial Pressure, HR=Heart Rate, BPM=Beats Per Min, * = P < 0.05, # = P < 0.01, § = P < 0.001. Number of experimental units = 12.
Figure 3. Coefficient of variation (CV) of the MAP and HR for F344 (A and B) and BN (C and D) rats with three cage items and control groups during the dark and light phases. Abbreviations: MAP=Mean Arterial Pressure, HR=Heart Rate, # = P < 0.01, § = P < 0.001. Number of experimental units = 16.
Table 1. P-values for significant mean arterial pressure (MAP) and heart rate (HR) coefficient of variation (CV) comparisons between the groups and corresponding point estimates (PE) for both F344 and BN rats and both light phases on observation days. Comparisons with no significances are excluded from the table. NS = not significant.

<table>
<thead>
<tr>
<th>Day</th>
<th>Diet board/Control</th>
<th>F344 rats p &lt; / PE</th>
<th>BN rats p &lt; / PE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MAP dark</td>
<td>MAP light</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Plain board/Control</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Day 10</td>
<td>Diet board/Tube</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Diet board/Control</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Day 14</td>
<td>Tube/Control</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

significantly (P < 0.01 - 0.05) lower in the diet board group compared to rats in the plain board groups (Figure 2d). However, none of these significant HR comparisons reached the threshold of biological significance.

The MAP CV of the control BN rats was significantly (P < 0.001 - 0.01) lower than in both board groups, both in the dark and light phases of day two. During the second week of the study period, the MAP CV was significantly (P < 0.01) higher in the diet board group on day ten in the dark compared to the controls and tube groups (Figure 3c). In the BN rats, the HR CV exhibited no significant results. All possible comparisons are illustrated graphically in Figure 3 and those which are statistically significant with corresponding point estimates in Table 1.

Corticosterone and IgA assays. The number of faecal pellets collected varied from none up to more than ten pellets per animal. Neither of the studied rat strains exhibited significant differences in amounts of corticosterone nor IgA excreted via faeces between the furniture item groups. However, the F344 rats had significantly (P = 0.05) higher faecal corticosterone outputs than BN rats whereas the excreted amounts of faecal IgA were significantly (P < 0.05) higher in the BN rats (Figure 4). In neither strain were any significant faecal assay CV differences found.
Figure 4. Corticosterone (A) and Immunoglobulin A (IgA, B) excreted via feces in F344 and BN rats with three cage items and control rats. Values expressed as cage means ± SEM nmol corticosterone or μg IgA excreted per hour per kg body weight. Number of experimental units = 16. The F344 rats displayed significantly (p = 0.05) higher fecal corticosterone levels than BN rats, while the opposite was true for fecal IgA levels (p < 0.05).
Table 2. Comparison of cage types: mean MAP and HR in F344 and BN rats. Arrows show the cage type with higher value. * < 0.05, ** < 0.01, *** < 0.001

<table>
<thead>
<tr>
<th></th>
<th>F344 MAP</th>
<th>F344 HR</th>
<th>BN MAP</th>
<th>BN HR</th>
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<tbody>
<tr>
<td>Dark</td>
<td>ns</td>
<td>OPEN ↑***</td>
<td>ns</td>
<td>OPEN ↑***</td>
</tr>
<tr>
<td>Light</td>
<td>OPEN ↑***</td>
<td>OPEN ↑***</td>
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<tr>
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<th>BN MAP</th>
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<td>OPEN ↑***</td>
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<tr>
<th></th>
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<th>F344 HR</th>
<th>BN MAP</th>
<th>BN HR</th>
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<tr>
<td>Day 10</td>
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<th>F344 HR</th>
<th>BN MAP</th>
<th>BN HR</th>
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<td>OPEN ↑**</td>
<td>ns</td>
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<tr>
<td></td>
<td>Light</td>
<td>OPEN ↑**</td>
<td>OPEN ↑***</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns = not significant

Discussion
The telemetry study of Sharp et al. (2005) assessed the outcome from an enrichment program on heart rate (HR), systolic blood pressure and activity. It concluded that lower HR in SHR rats was attributable to their enrichment program, but with SD rats no effect was detected. Their housing refinement items were a combination of several smaller items added to the cage at a few days intervals.

Irrespective of the housing system and enrichment program, Wistar rats seem to be more active in the dark phase than in the light phase, and they rest and sleep more in an enriched environment (Batchelor, 1994). In the present study, the diurnal variation of LA in F344 rats was similar. On day ten during the dark phase, the control group LA was lower than in the groups with furniture items without restricted feeding (Figure 1). When rats were housed in individually ventilated cages (IVC), the highest LA on day ten was also found in the tube group (Kemppinen et al., in press), but in both cases, between groups LA differences had disappeared by day 14.

In the study of Kemppinen et al. (in press) the same methodology and the same animals, albeit younger, as in the present study were used to evaluate the effect of furniture items in IVCs. In the IVCs, the F344 rats with the tube exhibited higher values of MAP compared to both board groups throughout the two-week period. In the conventional cages, the same was observed only on day 14 (Figure 2a). A major difference between conventional and IVCs was observed in the BN rats; rats in
the IVC displayed small, 1-2 mmHg, MAP differences between the groups, while in the open cages they were as much as 6 mmHg (Figure 2c), and additionally MAP levels of all rats in groups with furniture items were below the values of the controls.

When the two types of cages are compared, the group MAP differences of the F344 rats were smaller in the conventional open top rat cages (Figure 2a) than in IVCs, but in BN rats the situation was opposite (Figure 2c) (Kemppinen et al., in press). Nonetheless, overall LA and HR differences between the strains were of the same magnitude in the open top rat cages and IVCs. The CV for the MAP and HR in BN seemed larger in open cages than in IVCs, whereas in F344 rats CVs appeared to be about the same amplitude (Kemppinen et al., in press).

The IVC has become a common housing system for laboratory rodents. However, it is not always appreciated that the physical environment inside the cages may be very different from that of conventional cages in the same room. Indeed, differences have been found in illumination, sound level, temperature and RH (Kemppinen et al., 2008b). The higher sound level due to ventilation in the IVCs may affect the behaviour of the rats, while animals in the open cages are more likely to hear noises originating from care routines (Voipio et al., 2006), and research procedures.

Even small changes in ambient temperature can have an impact on cardiovascular parameters in rats; when temperature increases, MAP and HR of SD female rats decreases (Swoap et al., 2004). This effect was seen in HR of this study throughout the study; both rat strains had significantly higher HR in the open cages (Table 2) compared to the IVCs, where the temperature was 1-4 °C higher (Kemppinen et al. 2008b).

However, in the present study the rats were eight weeks older than in the IVC study and perhaps the differences cannot be attributed to cage type alone. Zhang & Sannajust (2000) reported a decrease in the nocturnal HR in old Wistar rats, an apparently opposite finding to our study, with higher HR in older rats with both strains. This may be due to the fact that the old Wistar rats were two years old, in contrast to the 8-10-months old rats examined here.

Rats are nocturnal animals and blood pressure and HR are elevated during the night (Sharp et al., 2005; Zhang & Sannajust, 2000; Lemaire et al., 1995; van den Brant et al., 1999). Many studies have described considerable differences in basal blood pressure and HR between different rat stocks or strains. This study used two rat strains, F344 and BN, for enhanced precision and applicability (Festing et al., 2002). These strains differ in various aspects of physiology, e.g. systolic and diastolic blood pressure, HR (van den Brant, 1999), plasma corticosterone (Armario et al., 1995; Sarrieau & Mormède, 1998), and brain and pituitary mineralocorticoid receptor levels (Gómez et al., 1998; Marissal-Arvy et al., 1999). The F344 and BN rats exhibit differences in level and diurnal rhythm of locomotor activity (Kemppinen et al., 2008a; van den Brant et al., 1999; Ramos et al., 1997), and in behaviour (Spangler et al., 1994; Rex et al., 1996; van den Staay & Blokland, 1996).
There was a significant group*strain interaction in MAP and HR on nearly every day examined during the two-week study period demonstrating strain differences. The F344 rats exhibited higher blood pressure and HR than BN rats with all items and the same was true for LA of the F344 rats during the dark phase (Figure 1). Van den Brant et al. (1999) detected a similar trend in telemetrically measured systolic and diastolic blood pressure, HR and night activity. They concluded that the BN rats have lost the typical night activity pattern typical for rodents. The present study shows that the F344 rats had considerably larger night-day difference both in the MAP and HR values than the BN rats. Presumably pigmented BN rats do not avoid light to the same extent as the albino F344 rats do. Albino rats prefer a cage with a shelter to one without (Patterson-Kane, 2003; Townsend, 1997) and they tend to remain in the tube during the light phase (Eskola et al., 1999).

This study compared all MAP and HR between the furniture groups to the corresponding strain-specific night-day differences of the controls. We suggest that when between-the-group differences are smaller than the night-day differences, a result displaying statistical significance is not biologically important (Kemppinen et al., in press; Kemppinen et al., 2009). On this basis, none of the F344 rats’ cardiovascular parameter differences between the furniture items were biologically meaningful, i.e. they should be considered as below the threshold of reliable differences (Figures 2a-b). In the earlier report with similar furniture in IVCs, both boards were superior to the tube (Kemppinen et al., in press). The trend seen in the present study on day 14 was similar, but due to increased night-day MAP difference, it lacked biological significance. No habituation to the furniture items was detectable in this study.

Even if in the BN rats there wasn’t any the only biologically significant result was observed on day 14 in the light phase in MAP, when the rats had the lowest value in the groups with the boards. Moreover, on all recorded days and both light phases, the boards and also tube MAP values were in most cases significantly lower than corresponding control values (Figure 2c). We conclude that in BN rats all furniture items tested were beneficial, and the boards were superior to the tube.

Krohn et al. (2003a) reported that systolic blood pressure and HR in rats increase by 6-7% when rats are housed on grid or solid bottom floors compared to solid floor with bedding, but they concluded that these differences may not possess biological value although they may be statistically significant (Krohn et al., 2003b). We suggest that a constant percentage as a cut off level for what is biologically relevant or not may not be applicable to all strains. Strain-specific measures, such as night-day difference are more valid for the purpose of determining what is biologically meaningful and what is not.

In CV of F344 rats there was only one significant finding; day 14 light phase HR CV of the tube group was higher than that in the controls (Figure 3b). The corresponding point estimates showed that with the tube, the number of animals needed would be 1.56
times that of controls when HR is the result parameter (Table 1). The BN rats had higher MAP CV in the boards compared to the controls on day two in both light phases (Figure 3c). The same was seen in F344 rats in the IVCs (Kemppinen et al., in press), and this may be due to novelty effect of the item introduced into the cage on the previous day. The point estimates in the BN rats were 1.87 - 2.25 and throughout the study they were higher in the open top than in the IVCs (Kemppinen et al., in press) and they were higher compared with the F344 rats as well (Table 1). It seems that the BN rats have larger variation in the open cages than in IVCs. These results and those from our previous study show that cage furniture has strain specific consequences on within-group variation and hence on number of animals needed in blood pressure studies.

Ferrari et al. (1987) reported that a cholinergic blockade in rats results in 30 % reduction in HR CV and an increase in MAP CV, but that a reduction in HR CV induced by sympathetic blockage is not accompanied by a change in MAP CV. Similarly to the IVC results (Kemppinen et al., in press), in the open cages there were only minor HR CV changes in both strains and none of them conformed to the scheme proposed by Ferrari et al. (1987) It may be that rats require more challenging stimuli than those resulting from the furniture items used. Moreover, it has to be borne in mind that results of Ferrari et al. (1987) were seen in a situation where a part of autonomic nervous system was blocked.

In this study no differences in faecal corticosterone or faecal IgA could be attributed to cage furniture. Eriksson et al. (2004) have shown that the proportion of the corticosterone and IgA excreted into faeces and urine is at its highest during the dark phase. Other studies have also shown the same to be true with faecal corticosterone, (Bamberg et al., 2001; Lepschy et al., 2007; Pi hl & Hau, 2003, Royo et al., 2004) and faecal IgA (Royo et al., 2004). Royo et al. (2004) stated that stress-induced changes in excreted IgA concentrations are slower than changes in corticosteroids and consequently faecal IgA may be more useful for assessing long-term well-being while faecal corticosterone is better at monitoring acute stress events. The finding that F344 rats have higher corticosterone and lower IgA excretion into faeces than BN rats, supports the overall concept that F344 strain is the more stress prone of the two strains.

Moncek et al. (2004) reported higher circulating corticosterone levels in Wistar male rats housed in enrichment cages compared to the non-enriched cages. However, comparisons to this study are complicated, because they used a combination of objects as enrichment; toys, tunnels, swings and running wheels and the enriched cage was more than twice the size of the control cage. Furthermore, the enriched cage had ten rats and the control cage 3-4 rats; but nonetheless cage density was lower in the enriched cages. Krohn & Hansen (2002) suggested that corticosterone may have only limited value for assessing the effects of small environmental changes on laboratory rodents. The present study confirmed that F344 rats have higher circulating corticosterone levels than BN rats (Sarrieau & Mormède, 1998), (Figure 4a). Siswanto et al. (2008) have
shown that there needs to be quite substantial changes in serum corticosterone for these to be detectable in faeces, and the HPA-axis may not be stimulated enough by changes in cage environment to be seen in faecal corticosteroid excretion.

In the present study, two rat strains, F344 and BN, were used to achieve the better presentation for the rat as a species (Festing, 2002), and as the results showed that the strains did not respond to the cage items equally and they responded differently to tube and dividing boards. These findings show that the environment effects to the study results in rats. The study of Richter et al. (2009) argues that genetic and environmental variations cause the poor reproducibility of experimental outcomes and thus, the environmental standardization can contribute to spurious and conflicting findings and unnecessary animal use. However, environment and animals used in the study are controlled to a large extent by the researcher, otherwise than the random variability, like inter-individual differences (Festing, 2002).

In summary, cardiovascular parameters are more sensitive than faecal corticosterone and faecal IgA for assessing the physiological impact of various types of cage furniture. Based on the MAP results for F344 rats, the tube appeared to be a poor choice for cage furniture, while in BN rats all furniture items seemed beneficial, but both the board types were superior to the tube. Cage furniture may result in increasing variation in the physiological parameters studied and may thus increase the numbers of animals needed in blood pressure studies, albeit a lack of consistency in the results were obvious in the present study. In conclusion, it may be futile to aim for general guidelines for optimal cage furniture in terms of environmental enrichment for laboratory rats due to their genetic variation in responses, and the wide variety of housing systems in different laboratory animal facilities.

Acknowledgements
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CHAPTER VII

GENERAL DISCUSSION
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7.1 IVC vs. open cage

IVC systems provide protection against animal infections and confer occupational benefits for personnel; this latter effect through a reduction in the levels of ammonia and airborne allergens (Keller et al. 1983, Lipman et al. 1992, Renström et al. 2001, Teixeira et al. 2006). It is generally believed that the IVCs and open cages share the same physical environment whenever they are in the same room. This study found that this is not the case, but that they represent rather different physical environments; the magnitude of the difference being large enough to have impact on the animals inside the cages. Table 7.1 presents a summary of physical cage environment between the caging systems.

Due to the additional cover, the light intensity in IVCs was lower than in open top cages. Not surprisingly the brightest cages were on the top row of both racks and the dimmest on the bottom. The placements of fluorescent tubes on the ceiling were decisive in determining the light intensity in the cages; cages closer to light source were brighter within both cage types. Similar illumination results have been reported Clough et al. (1995) in transparent, individually ventilated cages; while in the translucent, conventional cages, the illumination level was higher than in the cages used in this study. The dimmer lightning in the present study may be due to the black plastic sheet that was placed on top of the open top cage racks to equalize lighting in both cage types. In some conventional cages examined in this study, the lighting may have been too bright (see Chapter II), even to the extent that over a longer period it could have caused retinal damage in albino rats (Gorn & Kuwabara 1967, Stotzer et al. 1970, Weisse et al. 1974).

Since sound levels went down when approaching 16 000 Hz; it is fair to assume that no ultrasounds (> 20 000 Hz) are emitted into either cage type. Accordingly, the measured R-weighted sound pressure levels monitor accurately the sound level as heard by the rats. In open top cages, the R-weighted sound levels were about 7 dB(R) lower than in IVCs; i.e. loudness was about half and energy-wise the difference was fivefold. This different sound environment between the cages is presumably due to the extra lid and air fans of the ventilation machine in the IVC-racks. The difference in sound levels audible to the rat (R-weighted) appears to be large, yet it remains unclear whether the levels measured (< 25 dB(R)) have any impact on the animals.

The climatic conditions in the cage depend on those of the surrounding room as well as on the air supply to the cage-rack (Scheer et al. not dated). In the present study, when there were animals in the IVCs, this increased the temperature by 3-4 °C and RH by about 6 % in the cages, while temperature and RH in IVCs were the same as in the animal room when measured without animals. However, temperature and RH in the IVCs (Table 7.1) did not exceed the limits of the European
Table 7.1 Physical environment characterization of IVCs and open top rat cages in the present study.

<table>
<thead>
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<th></th>
<th>IVC</th>
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<tbody>
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<td>Top row: 53.8-91.0 lx</td>
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<tr>
<td></td>
<td>Bottom row: 3.8-4.9 lx</td>
<td>Bottom row: 14.3-25.3 lx</td>
</tr>
<tr>
<td>Sound level in empty cage</td>
<td>20-25 dB(R)</td>
<td>12-18 dB (R)</td>
</tr>
<tr>
<td></td>
<td>45-47 dB(A)</td>
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<tr>
<td>RH (3 rats in cage)</td>
<td>39-65 %</td>
<td>35-67 %</td>
</tr>
</tbody>
</table>

guidelines (Council of Europe 2006). Swoap et al. (2004) showed that even small changes in ambient temperature can alter cardiovascular parameters in rats; when temperature increased, MAP and HR of SD female rats decreased. This effect was seen in HR of the present study throughout the study; both rat strains had significantly higher HR in the open cages than in the IVCs, where the temperature was 1-4 °C higher. However, in the open cages, the rats were eight weeks older than those in the IVCs, hence the differences cannot be attributed to the cage type alone. Zhang & Sannajust (2000) reported a decrease in the nocturnal HR in old Wistar rats, an apparently opposite finding to this study, with higher HR in older rats with both strains. However, their old Wistar rats were two years old, whereas our rats were only 8-10-months old in the open top cages.

The rats used of this study were older than the types of young adult animals most commonly used in basic biomedical research. However, now than genetically altered rat strains are becoming more popular, this age class will become increasingly common in many facilities in addition to those used in long term safety studies. It is known that both BN and F344 rats develop spontaneous lesions as they grow older; BN rats in adrenal glands, kidneys, lungs and pancreas and F344 rats in eyes, heart, lungs and kidneys (Lipmann et al. 1999), none of these problems were seen in our rats during the 16 week study. This is not surprising since these lesions typically occur in rats older than those examined here.

The similarity of the temperature and RH in the open top cages and in the animal room is in line with the results of Clough et al. (1995), who essentially used the same type of IVCs as ours in terms of physical environment and compared them to rats housed in conventional cages. Also Reeb et al. (1998) have reported lower temperature and RH in empty control mouse IVC when compared to room air. Moreover, microenvironment temperature and RH in mouse IVCs are known to be significantly higher with lower ventilation rates, such as 30-40 air changes/h (Reeb et al. 1998). This shows that IVC-ventilation is not capable of removing excessive heat produced by the animals and heat generated from urine-faeces-bedding fermentation. The fermentation reaction is an unlikely candidate for heat generation because ventilation tends to keep bedding quite dry. However, the heat emission from one animal
has been estimated to be about 4 W (Heine 1998), and this is a likely source of excessive heat in the cage.

It appears that the transition from traditional open top cages to IVCs may cause changes in the physical environment, especially if the incoming air into the IVC-system is drawn from the room air. This makes any comparisons between these caging systems problematic without characterization of the physical parameters e.g. lighting intensity, sound environment, temperature and RH. In the present study, several differences were found in the responses of rats housed in IVCs and open-top cages; these will be discussed later in this chapter.

7.2 Diet board as a restricted feeding method in rats

In the present study, the rats with the diet board grew less than those on ad libitum feeding in both cage types. The diet board worked especially well with the F344 rats in controlling body weight. The F344 rats with the diet board even lost weight in the open cages, when they were older, but the magnitude of loss was marginal; only a few grams during two weeks, most likely fat tissue. The rats ate 12 - 18 % less with the diet board as compared to the respective controls. The same was seen in a study with the outbred Wistar rats; with the diet board they ate significantly less and had lower body weights than ad libitum feeding controls (Kasanen et al. 2009a).

The rats in the open cages ate more with the plain board than with the two other controls - apparently because the plain board round followed the diet board round, and the animals regained their weight loss in that round. Nevertheless, the diet board seems to be a better restricted feeding method than the “foraging device” used in the study of Johnson et al. (2004); the weight gain of the rats was higher with the foraging device than that encounters in ad libitum fed controls, and when the rats had limited access to food, their body weights remained unchanged, both indications of method failure.

Other ways to decrease the weight gain of rats have been tried; e.g. sugar beet pulp fibre made from water soluble polysaccharides (Eller et al. 2004). In that study, the rats grew less when fed with the fibre diet, but an autopsy after the study revealed an enlarged digestive tract in the rats that had received the fibre enriched diet, especially the caecum was enlarged. This may have been caused by hygroscopic effect of the fibre.

In early studies it has been demonstrated that rats are willing to work for food; if the rats had free access to food they would rather earn their food as long as the work demands were low (Carder & Berkowitz 1970, Neuringer 1969). Johnson et al. (2004) also reported that the rats preferred to eat mostly from the foraging device which required digging gravel in order to gain access. This may reflect the rats’ need to perform foraging behaviour as they would in their natural environment.

In the present study, the rats had to gnaw the wood if they wished to eat the food pellets from the diet board. Thus, the rats gnawed the wood most with the diet board as compared to plain board and tube groups in both cage types. The F344-rats gnawed wood significantly more than the BN rats - these rats hardly gnawed any wood from plain board and tube - this may related to a difference in the natural behaviour of these two strains (Rex et al. 1996, Ramos et al. 1997). Eskola et al.
(1999) showed that outbred Wistar rats would spontaneously gnaw aspen blocks and tubes but this opportunity for gnawing combined with ad libitum feeding had no effect on their growth; a similar situation to F344 rats with plain board and tube. Sørensen et al. (2004) have suggested that excessive gnawing in rats is related to escape or frustration – in the present study in the F344 rats, the gnawing could be more related to escaping.

In both cage types, the F344 rats were significantly more active in the dark compared to the BN rats with all cage items. Since there were no differences in the activity between the plain board and diet board groups, we conclude that work associated with the diet board was not overly laborious to the animals. Furthermore, the activity of the rats that had to work for food was no different from their activity during ad libitum feeding. In previous studies, it has been shown that rats with limited access to food spend more time feeding, but with the “foraging device” the time spent feeding was markedly decreased (Hawkins et al. 1999; Johnson et al. 2004). There were no significant changes in the total activity levels between the groups. In the rat groups, there were no changes in the social hierarchy nor any increased fighting or stereotyped behaviour when rats had limited access to food (Hawkins et al. 1999).

Rats consume most of their food during the night; Spiteri (1982) reported that 94% of food intake takes place during the dark. A rat’s typical feeding rhythm shows two peaks in the dark phase, the first peak at the beginning of the dark and the other at the end of that period (Spiteri 1982; Strubbe et al. 1986). Light shifts the clock in a circadian time-dependent way ensuring entrainment according to the illumination cycle. Eating activity, similarly to the other day-night rhythms is controlled by the circadian oscillator located in the suprachiasmatic nuclei. It has been suggested that there are other oscillators involved in the circadian system and this accounts for the flexibility needed for adaptation to different external and internal stimuli (Anglés-Pujolrás et al. 2006).

If the rats are given meals for a few hours daily, the food is eaten almost instantaneously; this impairs their natural feeding activity and consequently the associated gastrointestinal physiology. The diet board used for food restriction in the present study allowed rats to retain a natural feeding pattern and the feeding activity was similar to that encountered with the plain board. Kasanen et al. (2009a, 2009b) have also shown that when rats have access to the diet board, they maintain their normal diurnal eating rhythms. Furthermore, the diet board appears to result in higher serum corticosterone and decreased IgA levels in rats compared to the ad libitum feeding controls, yet no obvious stress pathology was associated with its use (Kasanen et al. 2009b).

7.3 Cage change and IG-gavage

The results of the present study show that cardiovascular responses to cage change and gavage can be modified by provision of the cage furniture items in both strains studied. Overall, there are very few previous studies on the impact of cage items to procedural responses. In most of the published studies on cage change or IG-gavage, the cage items used are either not mentioned or all the studied groups have had the same objects (Saibaba et al. 1996, Schnecko et al. 1998, Brown et al. 2000, Duke et al. 2001, Sharp et
The MAP and HR responses to gavage during the first hour after the procedure appear to be smaller in magnitude to those associated with the first cage change. In the study of Ökva et al. (2006), the immediate responses in blood pressure and HR to cage change and IG-gavage in outbred Wistar rats were essentially the same as those seen in F344 and BN rats in this study. Moreover, Seggie & Brown (1975) found a higher increase in the corticosterone level when rats were moved to a novel environment compared with short-term handling. The IG-gavage is a short-term procedure, commonly considered to be more stressful than handling. One common factor with both procedures is that the rats are returned back to the familiar home cage. In the cage change procedure, the animals are placed into a new environment with the new odours; hence it is no surprise that there are greater responses to cage change.

Immediately after the rats had been placed into clean cages, their activity increased. This finding agrees with the several other studies (Burn et al. 2006, Schnecko et al. 1998, Saibaba et al. 1996). The behaviour repertoire of the rats is also affected in clean cages; walking and skirmishing frequency increase immediately after the cage change, whereas grooming, eating, drinking, resting, rearing and bedding manipulation all decrease (Burn et al. 2006). The skirmishing or fighting of rats after the cage change is associated with dominance hierarchies within the group, and is related to their territory (Barnett 1958), e.g. a new cage. Being exploratory animals, rats investigate their new surroundings by ambulating and rearing (Hughes 1968) and these activities increase the cardiovascular parameters and LA after the cage change. The cage furniture can serve as an odour cue making the new cage environment more familiar to rats.

The cage change procedure increased MAP, HR and activity instantly in all study groups, but the values returned close to baseline within one hour. The same phenomenon was seen in the results of Duke et al. (2001) and Sharp et al. (2002, 2003), who also detected a notable elevation in blood pressure and heart rate after the cage change, but in these studies both parameters returned to baseline within 60-180 min. HR of the rats has been shown to increase only by moving the cage from the cage rack (Gärtner et al. 1980). Schnecko et al. (1998) reported that if the change took place in the morning, during the resting period, the systolic and diastolic blood pressure and HR responses were larger compared to situation if the change occurred during the activity period in the evening. A recent study of Abou-Ismail et al. 2008 reported that if rats experienced a cage change during the light period, they slept less and had more chromodacryorrhea, reduced thymus weight, increased aggression, and less object-directed behaviour. The late timing for husbandry procedures is unlikely to work in practice; hence in this study the procedure was carried out during afternoon within working hours.

In the present study, assessment on whether statistically significant differences detected possess biologic or welfare relevance, we utilized strain-specific reference values, the night–day differences in MAP and HR calculated for the control group. Based on this comparison, the statistically significant MAP or HR responses to both cage change and IG-
gavage for F344 rats in IVCs were not greater than the night–day MAP or HR difference in these parameters and thus they cannot be considered biologically meaningful. Therefore, a biologically valid effect attributable to cage objects is not apparent in the current study. In open-top cages, many statistically significant differences were detected in the responses to both procedures in the F344 rats, but again, none of them were large enough to achieve biologic significance.

In the light, when the BN rats had the plain board in IVCs, the MAP response to IG-gavage was lower compared to the other items. This statistically significant difference exceeded the BN-specific MAP night–day difference, thus being biologically meaningful, but this was not the case for corresponding HR differences. The BN rats showed no significant between the cage furniture item differences in HR after the cage change. The MAP response was less during the period from one hour after cage change until dark in BN rats with a diet board when compared to the controls. The new diet board may have allowed easier access to food than the old, gnawed board, thereby leading to the changes in MAP. In open-top cages, the only observed effect in the BN rats was during the first hour after the IG-gavage and with the tube.

A summary of the MAP and HR differences to the baseline during one hour after cage change and in response to gavage in F344 and BN rats is presented in Table 7.2. It shows that significantly higher differences were mostly seen in the IVCs after the cage change. The reasons for this phenomenon might be the rats’ younger age in the IVCs or the difference in the physical environment in the two cage types as discussed in Chapter II.

### Table 7.2 Comparison of cage types: MAP and HR difference to the baseline during one hour after cage change and IG-gavage in F344 and BN rats. Arrows show the cage type with the higher difference value. * < 0.05, ** < 0.01, *** < 0.001

<table>
<thead>
<tr>
<th></th>
<th>Cage change</th>
<th>IG-gavage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP</td>
<td>HR</td>
</tr>
<tr>
<td>F344</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet board</td>
<td>IVC ↑ ***</td>
<td>IVC ↑ ***</td>
</tr>
<tr>
<td>Tube</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Plain board</td>
<td>NS</td>
<td>IVC ↑ **</td>
</tr>
<tr>
<td>Control</td>
<td>IVC ↑ ***</td>
<td>NS</td>
</tr>
<tr>
<td>BN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet board</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Tube</td>
<td>IVC ↑ *</td>
<td>IVC ↑ ***</td>
</tr>
<tr>
<td>Plain board</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Control</td>
<td>NS</td>
<td>IVC ↑ *</td>
</tr>
</tbody>
</table>

NS = not significant
7.4 Cage furniture items and stress indicators in rats

7.4.1 Cardiovascular parameters (MAP and HR)

This study shows that the cardiovascular responses of F344 rats and BN rats to the added furniture items were not the same. The F344 rats in the IVCs had the highest MAP in the tube cages all through the two week period irrespective of whether lights were on or off, whereas in the open cages the same was seen only in the last day of the period. The BN rats displayed a considerable difference between open cages and IVCs; in the IVC they were very small, 1-2 mmHg, MAP differences between the groups. In the open cages, they were elevated up to 6 mmHg, and additionally MAP levels of all rats in groups with cage items were below the values of the controls.

Krohn et al. (2003a) showed that systolic blood pressure and heart rate in SD rats rises 6-7% in stressful housing conditions, and concluded that changes below this percentage in systolic blood pressure and heart rate may not be biologically significant despite being statistically significant (Krohn et al. 2003b). The results of the present study suggest that a fixed percentage may not be applicable to all strains, and that a strain-specific measure, such as the night-day difference may be more valid for that purpose.

Some scientists have stressed the effect of the cage items on the variation instead of mean values. Some studies suggest that the cage items or “enrichments” may increase variation, resulting in a higher number of animals needed in experiments (Eskola et al. 1999, Mering et al. 2001, Tsai et al. 2002, Tsai et al. 2003a). The studies of Tsai et al. (2002, 2003a, 2003b) showed that enrichment items had an effect on the coefficient variation (CV) of body weight, haematological data, organ weights and breeding index in mice. In our studies with rats and cage items, the CV has not been used before as an indication of the variation within the experimental groups.

In this study, the F344 rats in the IVCs had the highest CV of MAP in the plain board on the second day both in the dark and light phases, and this may reflect the novelty of the cage items. The point estimates (see Table 7.5) showed that with the plain board, the number of animals needed would be about 1.5 times greater than with the other items or even controls when the corresponding cardiovascular parameters were result parameters. Surprisingly, the number of animals needed to achieve the same results with the plain board would have been higher than with the tube although the MAP with the tube was at the highest levels in F344 rats. Hence, the high blood pressure in the F344 rats with the tube was not associated with wide variation. In the open cages, the F344
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Rats displayed only one significant finding on day 14 when the HR CV in the tube group was higher than that in the controls.

The BN rats in the IVCs exhibited higher CV values of both MAP and HR in the control group compared to the diet board and the point estimates were 1.45 and 1.68, respectively. At the end of the study period, the BN rats seemed to have adjusted to the use of the diet board and the variation was significantly lower compared to the controls.

In the open cages, the BN rats had higher MAP CV in the boards compared to the controls on day two in both light phases that may be due, as in the F344 rats in the IVCs, to the novelty of the item which had been introduced into the cage on the previous day. The point estimates of the BN rats in open cages were 1.87 - 2.25 and throughout the study; they were higher in the open top than in the IVCs, and they were higher than in the F344 rats as well. It seems that the BN rats display a larger variation in the open cages than in IVCs.

Table 7.3 Biologically meaningful telemetric changes attributable to cage furniture items compared to controls in the present study. Statistical results are shown in Chapters IV, V and VI.

<table>
<thead>
<tr>
<th></th>
<th>IVC</th>
<th>Open cage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F344</td>
<td>BN</td>
</tr>
<tr>
<td>Cage change</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>IG-gavage</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>All statistically significant results of MAP (V)</td>
<td>-</td>
</tr>
<tr>
<td>Day 6</td>
<td>All statistically significant results of MAP (V)</td>
<td>-</td>
</tr>
<tr>
<td>Day 10</td>
<td>All statistically significant results of MAP (V)</td>
<td>-</td>
</tr>
<tr>
<td>Day 14</td>
<td>All statistically significant results of MAP (V)</td>
<td>All statistically significant results of MAP (V)</td>
</tr>
</tbody>
</table>
These results show that cage furniture has strain specific consequences on within-group variation. The same was described in the studies of Tsai et al. (2002, 2003a, 2003b) with the different mouse strains and measurements of body weight, haematological data, organ weights and breeding index. Overall, cage items may result in increased variation in the cardiovascular parameters studied and may thus change the numbers of animals needed in those studies, albeit the lack of consistency in the results was obvious in the present study.

7.4.2 Faecal corticosterone and faecal IgA

This study found no differences in faecal concentrations of corticosterone or IgA attributable to cage furniture in either of the cage types. There are studies to show that the highest amounts of corticosterone and IgA are excreted into faeces and urine during the dark phase (Bamberg et al. 2001, Eriksson et al. 2004, Royo et al. 2004, Pihl & Hau 2003, Lepschy et al. 2007). It has been stated that stress-induced changes in excreted IgA concentrations are slower than changes in corticosteroids and thus faecal IgA may be more useful for assessing long-term well-being whereas the faecal corticosterone is better for monitoring acute stress events (Royo et al. 2004). We found that F344 rats in the open cages have higher corticosterone levels and lower IgA excretion into faeces than BN rats; this supports the overall concept that F344 strain as being more sensitive for experiencing stress and have higher circulating corticosterone levels than BN rats (Armario et al. 1995, Ramos et al. 1997, Sarrieau & Mormède 1998).

Moncek et al. (2004) found higher serum corticosterone levels in male Wistar rats that were housed in enriched cages than in non-enriched cages. However, comparisons of these findings to the present study are complicated, because they used a combination of various objects as enrichment i.e. toys, tunnels, swings and running wheels and the enriched cage was more than twice the size of the control cage. Furthermore, the enriched cage housed ten rats and the control cage had 3-4 rats; but nonetheless cage density was lower in the enriched cages. Rushen & de Passillé (1992) have criticized the use of corticosterone as an indicator of animal welfare in different housing methods since they are not always closely related to the mental or emotional states of animals and the measures of a single hormone ignore the complex physiological reactions of animals to their environments. Furthermore, the study of Krohn & Hansen (2002) suggests that corticosterone may have only limited value for assessing the effects of minor environmental changes on laboratory rodents. Furthermore, Siswanto et al. (2008) showed that there needs to be quite substantial changes in serum corticosterone for these to be detectable in faeces, and the HPA-axis may not be stimulated enough by changes in cage environment for this to be reflected in changes in the faecal corticosteroid excretion.

In summary, cardiovascular parameters appear to be more sensitive than faecal corticosterone and faecal IgA for assessing the physiological impact of various types of cage furniture. Based on the MAP results for the F344 rats, the tube appeared to be a poor choice as a piece of cage furniture, while in BN rats all furniture items seemed to be beneficial, but both board types were better than the tube. Cage furniture may result in increasing variation in the physiological
parameters studied and may thus change the numbers of animals needed in blood pressure studies, albeit the lack of consistency in the results was obvious in the present study.

7.5 Differences in rat strains

In the present study, two inbred strains of rat, F344 and BN, were used to achieve greater precision and wider applicability of the results (Festing et al. 2002). These strains have been shown to differ in various aspects of physiology, e.g. systolic and diastolic blood pressure, HR (Van den Brant et al. 1999), plasma corticosterone concentration (Armario et al. 1995, Sarrieau & Mormède 1998) and brain and pituitary mineralocorticoid receptor levels (Gómez et al. 1998, Marissal-Arvy et al. 1999). Moreover, the F344 and BN rats exhibit different levels and diurnal rhythms in locomotor activity (Ramos et al. 1997, Van den Brant et al. 1999) and behaviour (Spangler et al. 1994, Van den Staay & Blokland 1996).

The results of this study confirm the results of previous studies with respect to blood pressure, HR and LA, but also that these strains have different food consumptions, weight gain and wood gnawing behaviour. In summary, BN and F344 rats responded differently to the cage furniture items and to the procedures. These and previous findings (Table 1.6 in Chapter I) in these two strains might be attributable to the activities of the rats with the items, and in the future, it would be interesting to study the behaviour of the rats with the same cage items. The summary of the results in F344 and BN rats is shown in Table 7.4.

The European regulations for laboratory rodents, although they are rather specific, are generally the same for all laboratory rodents, i.e. rats, mice, gerbils, hamsters, and guinea pigs (Council of Europe 2007). However, all rodent species and, as shown in this study, strains within a species, may have different needs. These differences raise the possibility that general guidelines, which may be valid for one species, may have a different effect on welfare in other species and strains.

The present study shows that for the F344 rats, the tube appeared to be a poor choice as a piece of cage furniture, while for BN rats all furniture items seemed to be beneficial, but both the board types were better than the tube. As with these two strains in two different cage types, it can be seen that there is a genetic component involved in rat responses to cage items as well. Sharp et al. (2005) also showed a difference between SHR and SD rats in their responses to procedures with a specified enrichment program, and the authors speculated on the difficulties of making generalized recommendations to the animal care community regarding rat enrichment programs. Thus, it may be necessary, on top of general guidelines, to add stock- and strain-specific modifications about optimal cage furniture for laboratory rats because of the obvious genetic component involved and also to be able accommodate the wide variety of housing systems in use.

7.6 The Two R value of the results

To achieve a better applicability towards the rat as a species, two defined rat strains (F344 and BN) were used in this study (Festing 2002), and the results reveal that the strains behaved quite differently. F344 and BN rats exhibit differences in LA, MAP and HR levels and in responses to the cage items and to the procedures. These findings suggest that that
Table 7.4 The summary of the results of F344 and BN rat strains used in the study.

<table>
<thead>
<tr>
<th></th>
<th>IVC</th>
<th>OPEN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>110.8 mmHg</td>
<td>92.7 mmHg</td>
</tr>
<tr>
<td>Dark</td>
<td>115.7 mmHg</td>
<td>95.6 mmHg</td>
</tr>
<tr>
<td><strong>HR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>335 bpm</td>
<td>284 bpm</td>
</tr>
<tr>
<td>Dark</td>
<td>388 bpm</td>
<td>309 bpm</td>
</tr>
<tr>
<td><strong>Activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>1.4 counts/min</td>
<td>1.0 counts/min</td>
</tr>
<tr>
<td>Dark</td>
<td>4.9 counts/min</td>
<td>2.2 counts/min</td>
</tr>
<tr>
<td><strong>FOOD CONSUMPTION, WEIGHT GAIN AND WOOD GNAWED</strong> (mean values)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Food consumed / 2 weeks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet board</td>
<td>712 g</td>
<td>607 g</td>
</tr>
<tr>
<td>Ad libium</td>
<td>874 g</td>
<td>612 g</td>
</tr>
<tr>
<td><strong>Weight gain / 2 weeks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet board</td>
<td>-1.5 g</td>
<td>7.6 g</td>
</tr>
<tr>
<td>Ad libitum</td>
<td>10.4 g</td>
<td>9.1 g</td>
</tr>
<tr>
<td><strong>Wood gnawed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet board</td>
<td>134 g</td>
<td>100 g</td>
</tr>
<tr>
<td>Plain board</td>
<td>16 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Tube</td>
<td>27 g</td>
<td>0 g</td>
</tr>
<tr>
<td><strong>RESPONSES TO THE CAGE ITEMS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAP difference (mean) to the controls at day 14 (Light/Dark)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet board</td>
<td>-5.6/-2.2 mmHg</td>
<td>-3.4/-2.2 mmHg</td>
</tr>
<tr>
<td>Tube</td>
<td>3.1/3.4 mmHg</td>
<td>-1.9/-0.4 mmHg</td>
</tr>
<tr>
<td>Plain board</td>
<td>-10.4/-5.9 mmHg</td>
<td>-0.2/-0.5 mmHg</td>
</tr>
<tr>
<td><strong>HR difference (mean) to the controls at day 14 (Light/Dark)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet board</td>
<td>-13.1/0.5 bpm</td>
<td>-13.9/-6.6 bpm</td>
</tr>
<tr>
<td>Tube</td>
<td>-1.2/-3.7 bpm</td>
<td>-9.4/1.7 bpm</td>
</tr>
<tr>
<td>Plain board</td>
<td>-112.9/-6.4 bpm</td>
<td>4.9/3.9 bpm</td>
</tr>
<tr>
<td><strong>Corticosterone and IgA (mean values)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosterone</td>
<td>NS</td>
<td>F344 had higher values (P = 0.05)</td>
</tr>
<tr>
<td>IgA</td>
<td>NS</td>
<td>BN had higher values (P &lt; 0.05)</td>
</tr>
</tbody>
</table>
all cage furniture items are not equally good for laboratory rats, irrespective of the strain, and that welfare indicators should be used to rank the feasibility of cage furniture.

The present study showed that conventional open cages and IVCs differ in temperature, RH, sound spectra level and light intensity. These findings did not appear to have any direct effect on the results of the studies made in those cages, but age difference of eight weeks between open cages and IVC do not allow simple comparisons. The European recommendations emphasize that enrichment has to be applied irrespective of cage system (Council of Europe 2006). Moreover, the applicability of any furniture brought into the cage requires that an item has a similar effect in all housing systems (Richter et al. 2009).

The current European guidelines on laboratory animal housing and care are similar for all rodent species i.e. rats, mice, guinea pigs, hamsters and gerbils (EU 2007). Within a species, they have to be implemented irrespective of animal strain, and caging system used. The results of this study indicated that it may be nesesary to devise strain specific guidelines for optimal cage furniture in terms of environmental enrichment for laboratory rats because of the genetic component involved in the responses of the animals to these items. Once strain specific guidelines for rats have been established, then it could be possible to see whether one can then devise species-specific guidelines. This is a labour intensive approach, but is there a scientifically valid shortcut within sight?

Environmental enrichment as a term refers to a positive impact on animals e.g. as based on biological functioning, behaviour and 2Rs (Chamove 1989, Newberry 1995, Purves 1997). The present study showed that unfortunately all items added to the cage do not result in a positive impact for rats; and indeed by definition environmental enrichment does not constitute enrichment before it is proven to have this affect. It is clear that all studies should carry detailed description of the cage furniture items used, and better designed studies are needed to rank various furniture items and other cage additions.

In animal welfare studies, it is essential to use a variety of welfare indicators to assess effects of animal housing and management systems. In the case if one parameter displays a negative impact to welfare, then the result is explicit, but if the result has a positive impact, more studies and more positive results are needed (Broom 1991, Clark et al. 1997). In the present study, four parameters (MAP, HR, faecal corticosterone and faecal IgA) were used to assess the animal welfare. To achieve an even more valid welfare impact of studied cage items, additional parameters should be added to the palette (e.g. behavioural and preference studies).

A recent study indicated that genetic and environmental variations are responsible for the poor reproducibility of experimental outcomes (Richter et al. 2009). However, the traditional opinion states that the wider range of genetic backgrounds and environments used in an experiment, the applicability of the results. This is exactly the reason why we used two different rat strains and two different caging systems to assess the welfare value of the cage items used.

Richter et al. (2009) stated that environmental standardization is indeed a
cause for weak reproducibility of study outcomes. Standardization of experiments has been claimed to reduce within- and between-experiment variation in order to facilitate detection of treatment effect and maximize reproducibility of results (Würbel 2000). The term standardization fallacy is used to describe the increase of reproducibility at the expense of external validity; i.e. how applicable the results are in some other environmental context, population and species (Würbel 2000). The study of Wolfer et al. (2004) showed that environmental enrichment does not disrupt the standardization; even though there are studies to suggest that complex cage environments may increase aggression among male mice (Nevison et al. 1999), which may increase variability in the aggressive strains of laboratory rodents (Festing 2002). In conclusion, it is questionable whether one can blame standardization for the poor results of reproducibility; on top of good standardization one has to emphasize applicability – in terms of variety of defined genetic backgrounds and environments used - as shown by Festing et al. (2002) and as used in this study.

This study used CVs and point estimates to assess between-group variation in F344 and BN rats. The summary of the point estimates reflecting the results from the corresponding significant CVs (p < 0.01) are presented in Table 7.5. The point estimates of day 2 showed that in the IVCs, the number of the F344 rats needed would be 1.36 - 1.81 times greater with the plain board than with the diet board, tube or control when the MAP was the result parameter, both in dark and light phases. On the other hand, in the open cages the point estimates of the MAP in the BN rats show that in the controls group, the number of animals needed would be 1.87 - 2.25 times higher than there needed in the diet board or plain board, a through 24 hour period of day 2. Since both of these findings were detected early in the two week period, this may be attributable to a novelty effect soon after the item had been introduced into the cage.

Some of effects on the point estimates carried over till the end of the fourteen day period. The use of the plain board meant also less animals would be needed than with the tube when MAP was the result parameter, but with HR the situation was reversed. The results showed that the number of animals needed could be reduced in blood-pressure studies. Although the results were strain specific and there were differences between the cage types, the cage furniture has a strong Reduction potential in biomedical studies.

In summary, the present study showed that the cage items and restricted feeding have Refinement and Reduction potential for laboratory rats. The diet board is a better method of restricted feeding compared to the previous methods; where rats have access to food only a few hours every day, or a certain amount of food is offered once a day. With the diet board, rats ate less and gained less weight in comparison to ad libitum-feeding, but it had no effect on daily feeding activity or other diurnal rhythms in rats. With respect to the use of the cage items, the cardiovascular responses to cage change and IG-gavage can be lessened and furthermore there is also an effect which lasts for weeks. In cardiovascular studies, number of animals needed can be reduced with suitable cage items. Rat welfare can be assessed with MAP and HR in relation to cage furniture, whereas changes induced by the items in faecal corticosterone and faecal IgA excretion seem too small to be
quantifiable. Based on the MAP results, the tube is not to be recommended as a cage furniture item for F344 rats, whereas the plain board and the diet board appear to be suitable for both strains.

Table 7.5 Point estimates for significant (p < 0.01) MAP CV and HR CV comparisons between the groups for both F344 and BN rats and for both light phases on the observation days. Arrow between the two cage items indicates the multiplier to be used for the number of animals needed for that transition direction.

<table>
<thead>
<tr>
<th>F344</th>
<th>IVC</th>
<th>OPEN</th>
<th></th>
<th>MAP</th>
<th>MAP</th>
<th>HR</th>
<th>HR</th>
<th>MAP</th>
<th>MAP</th>
<th>HR</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP</td>
<td>dark</td>
<td>light</td>
<td>HR</td>
<td>dark</td>
<td>light</td>
<td>MAP</td>
<td>dark</td>
<td>light</td>
<td>MAP</td>
<td>dark</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain board → Diet Board</td>
<td>1.36</td>
<td>1.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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7.7 Conclusions

The following specific conclusions were drawn from the results of this study:

1. Conventional open cages and IVCs differ in several physical parameters in the cage environment when IVC-system draws air from the room.

2. Rats eat less and gain less weight when fed with the diet board compared to *ad libitum*-feeding, and especially for the F344 rats, the diet board was an effective way to control weight.

3. Cardiovascular responses to both cage change and IG-gavage can be modified with the cage items in both studied strains.

4. The immediate MAP and HR responses to IG-gavage appear to smaller in magnitude than those associated with cage change.

5. Cardiovascular parameters can be used to assess the welfare value of cage furniture, whereas changes seen in faecal corticosterone and faecal IgA excretion would appear to be too small to be quantifiable.

6. F344 and BN rats have different LA, MAP and HR levels and different responses to the cage items and to the procedures.

7. The tube is not recommended as a cage furniture item for F344 rats, whereas the plain board and diet board are suitable for both strains.

8. It may be necessary to aim at strain specific guidelines for optimal cage furniture in terms of environmental enrichment for laboratory rats because of the genetic component involved in the responses of the animals to these items.
7.8 References


Rhee CK, Jones RB, Bearg DW, Bedigian H, Myers DD, Paigen B. 1998. Microenvironment in ventilated animal cages with different ventilation rates, mice populations, and frequency of bedding changes. Contemporary Topics in Laboratory Animal Science 37:43-49.


