

Refinement and Reduction Value of Aspen Furniture and Restricted Feeding of Rats in Conventional Cages

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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. In this crossover study, responses of all rats to the following cage furniture items were investigated: two types of simple maze, a rectangular tube and a control with no cage 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 furniture 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. Radiotelemetry as a method to record physiological parameters, such as HR and blood pressure, allows

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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 on 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 SHR 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 individually ventilated cage (IVC) system, and the rats exhibited habituation to some of the items (Kemppinen *et al.*, 2010).

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 4-12 hours later in faeces, after the 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 of corticosteroid metabolites 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 reduc-

tion potential of various cage 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 a study with the same methods as in the present study (Kempainen *et al.*, 2010).

Animal housing and care

Rats of the same strain were housed in 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 $21.2 \pm 0.3^\circ\text{C}$ (mean \pm SD) and the relative humidity (RH) $53 \pm 6\%$. 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 Kempainen *et al.* (2008b).

Cage furniture and study design

The experiment utilized a crossover *design* with two week periods and a rotational order, i.e all rats of each strain were housed for two weeks in each type of cage environment. There were two different kinds of cage 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 a control environment without any furniture. One divider type included drilled holes with 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 due to prior heating (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 for each group was randomly allocated. The illustration of the items and item order can be seen elsewhere (Kempainen *et al.*, 2009; Kempainen *et al.*, 2010).

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 cylindrical transmitter body (length 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 the 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 antennae, and the receiver detected signal strength change when the rat moved in relation to these antennae.

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 with 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 by 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 the second covariate. Significant CV differences between groups were processed further to point estimates [$= (CV_1/CV_2)^2$]. 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 for 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 cages 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 ($P < 0.001-0.05$) higher MAP 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 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, in both lighting phases the highest HR was recorded with the tube; with lights on the HR was significantly higher in the tube group ($P < 0.01$) compared to the plain board and in the dark compared to the diet board ($P < 0.001$, Figure 2b). Similarly to MAP, no HR comparison for F344 rats 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 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,

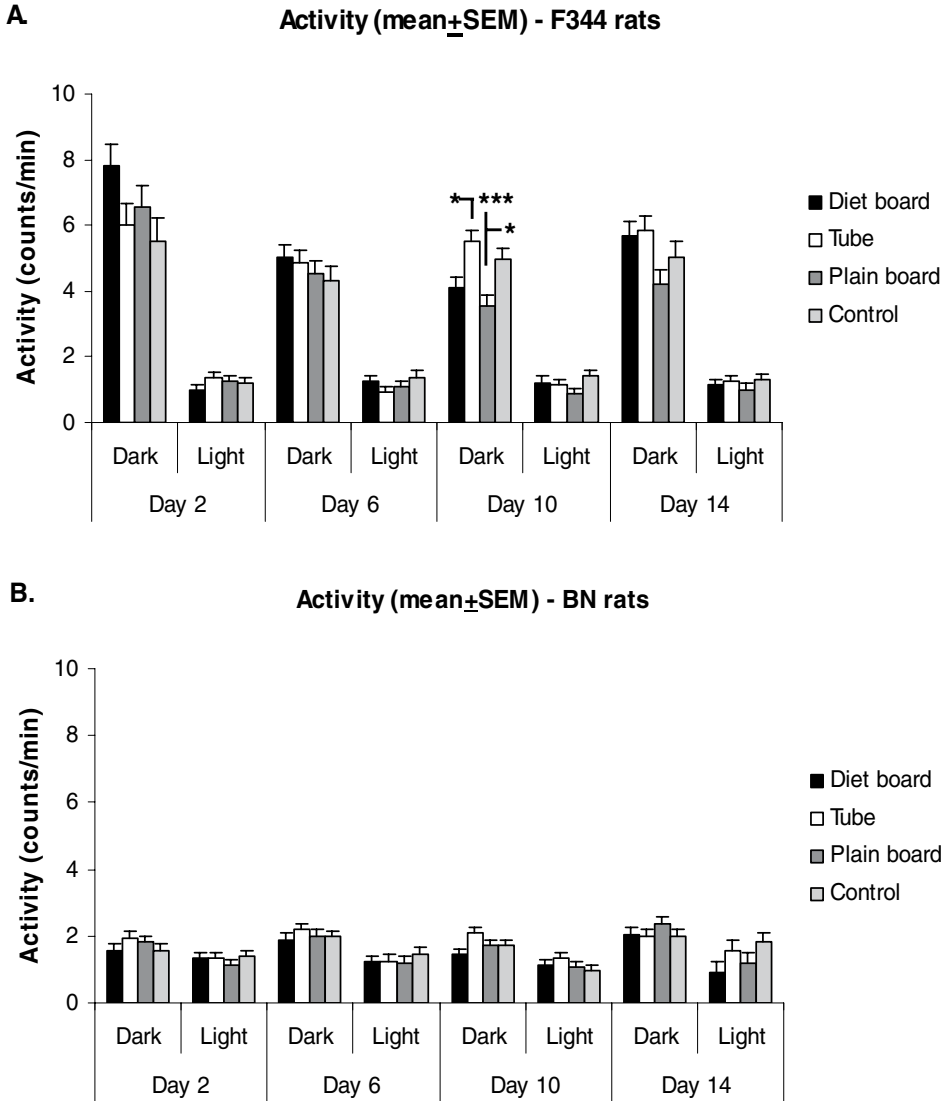
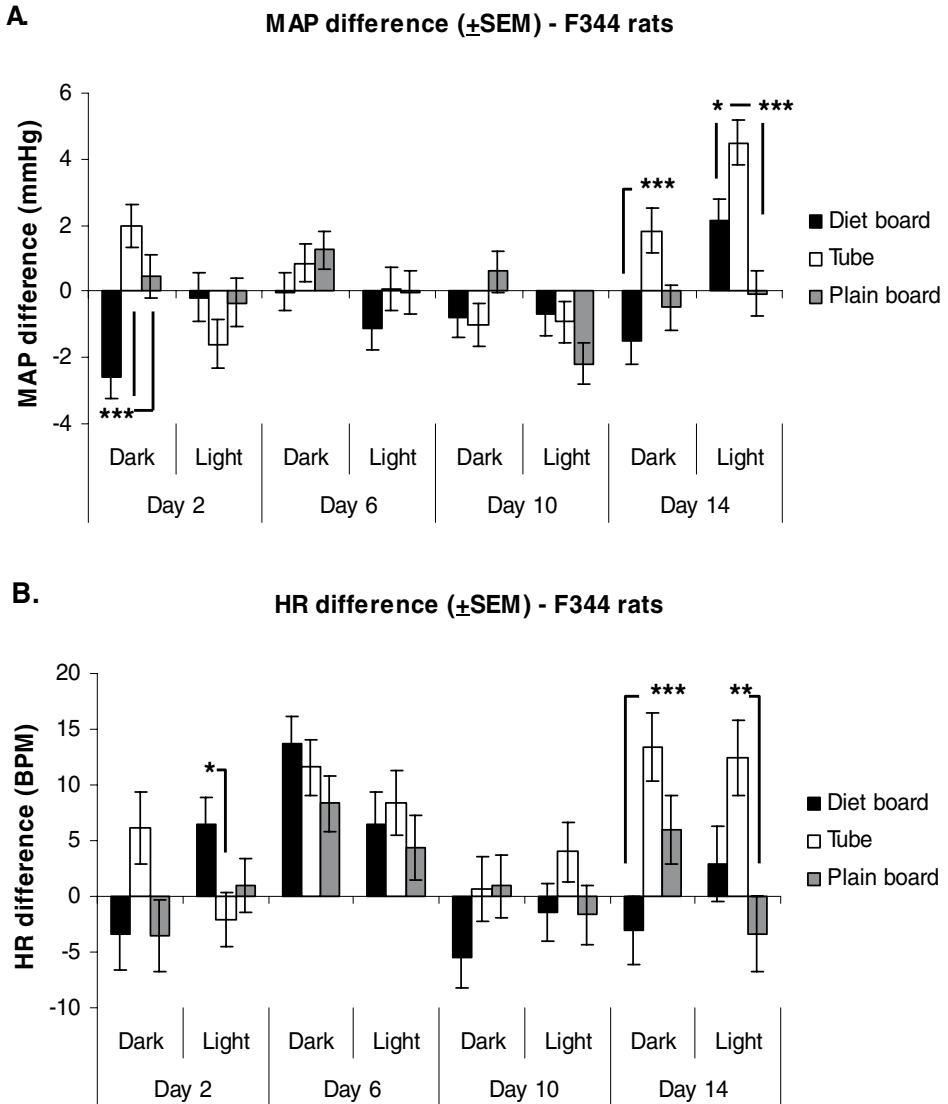


Figure 1. Locomotor activity (mean ±SEM) of F344 (A) and BN (B) rats with different cage items and controls during the dark and light phases. Abbreviations: * = P < 0.05, *** = P < 0.001. Number of experimental units within a strain = 16.

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 achieved significance (P

< 0.05); but this lowering effect compared to the tube group did not reach 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 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. When compared to control, MAPs for

all furniture item groups exceeded the biological significance threshold.

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

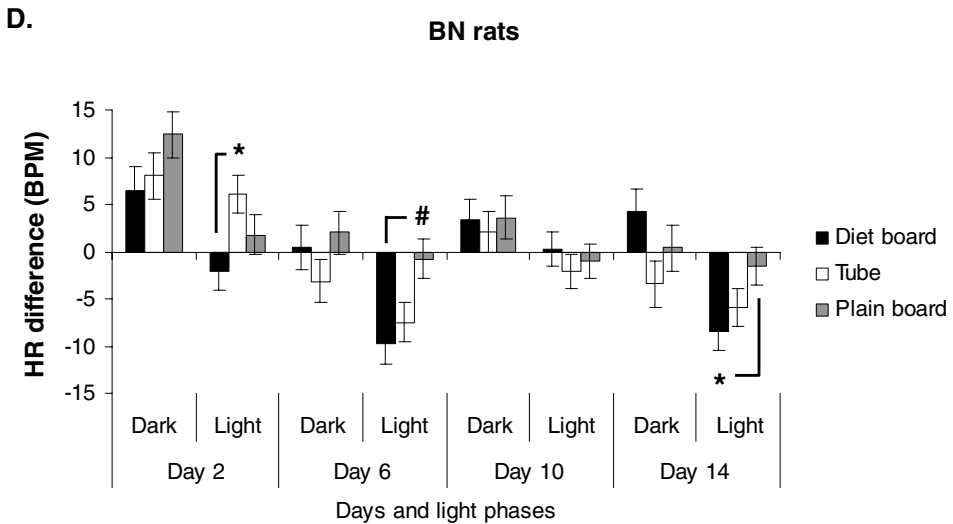
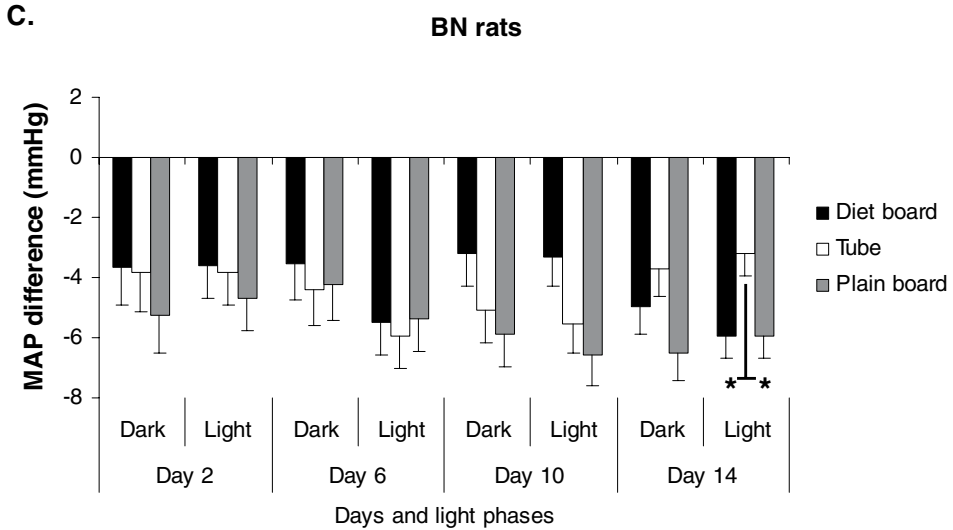
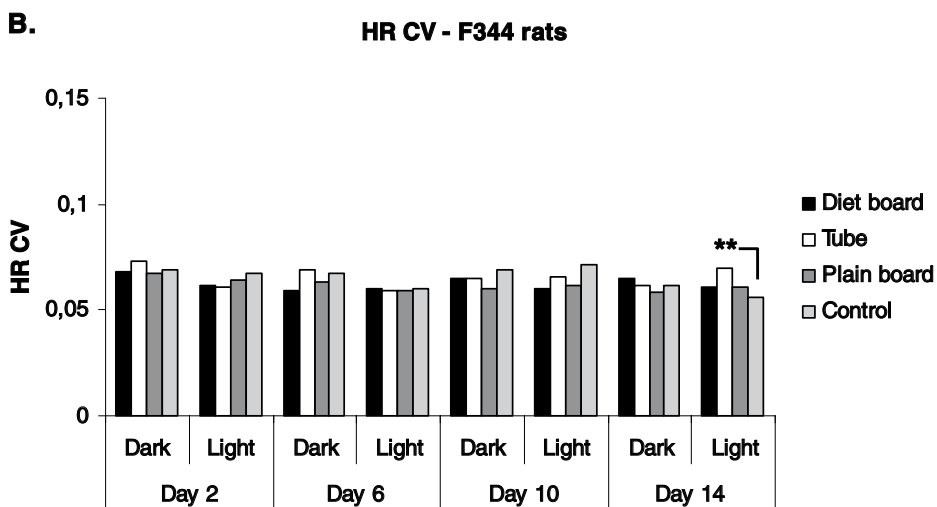
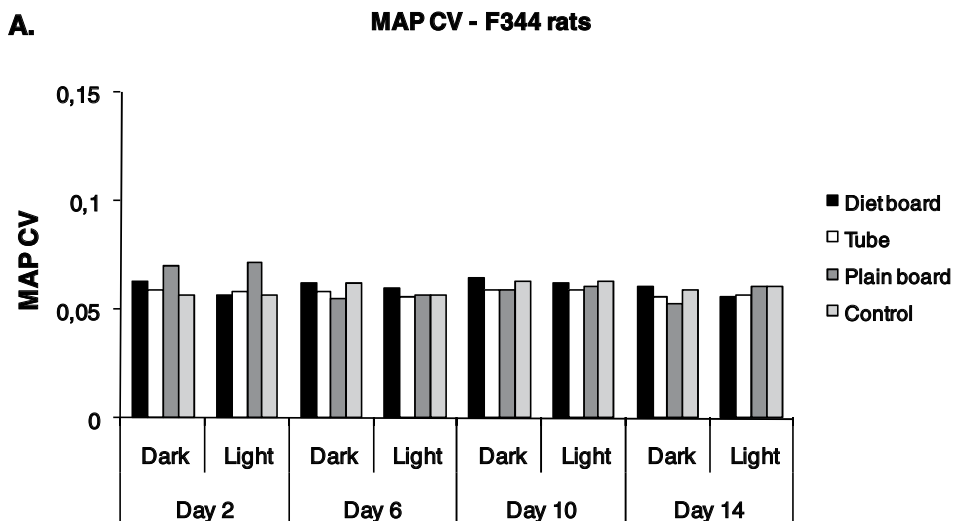


Figure 2. The MAP and HR differences (mean \pm SEM) of F344 (A and B) and BN (C and D) rats to controls with three cage items during the dark and light phases. Abbreviations: MAP=Mean Arterial Pressure, HR=Heart Rate, BPM=Beats Per Min, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Number of experimental units = 12.

diet board group on day ten in the dark compared to the control 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.

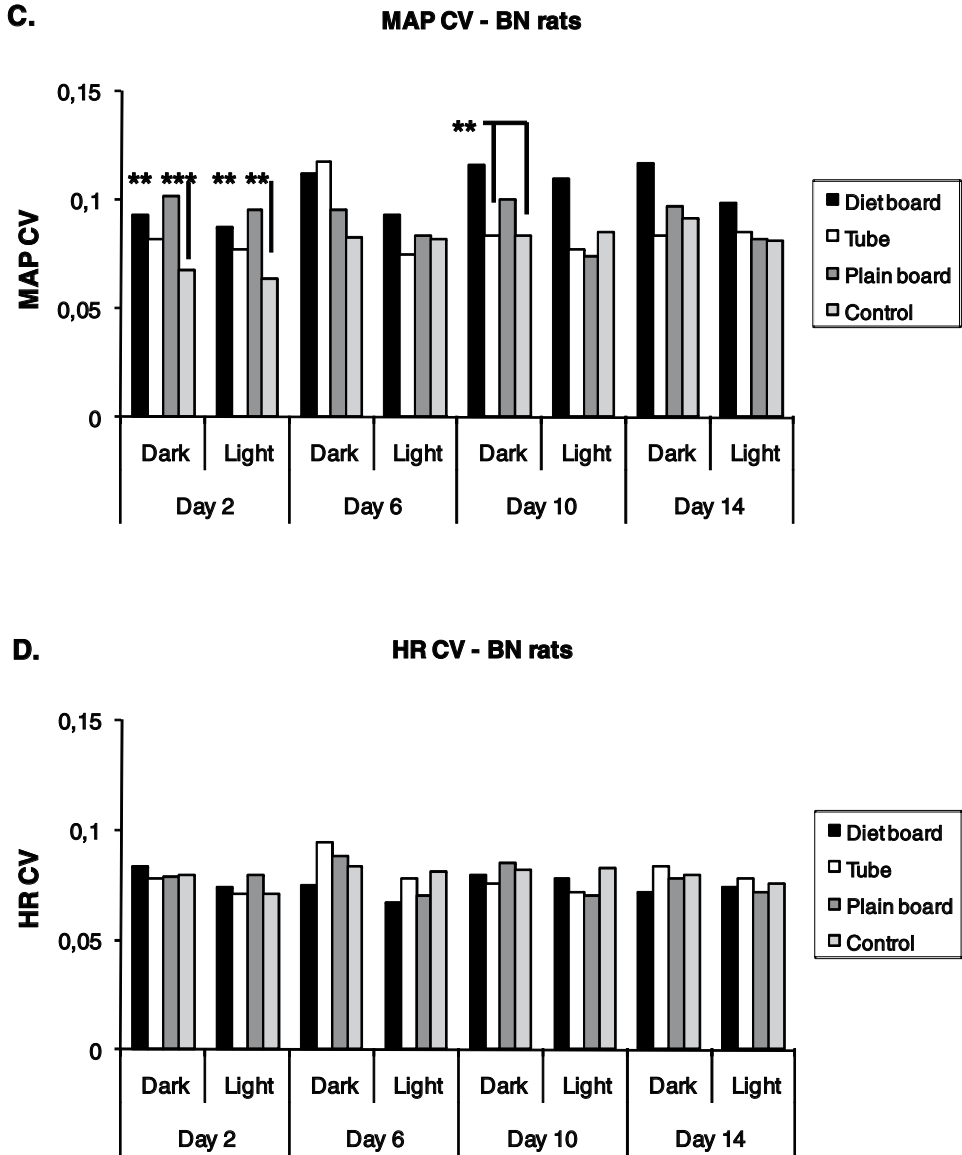


Figure 3. Coefficient of variation (CV) of the MAP and HR for F344 (A and B) and BN (C and D) rats with three cage items and control groups during the dark and light phases. Abbreviations: MAP=Mean Arterial Pressure, HR=Heart Rate, ** = P < 0.01, *** = P < 0.001. Number of experimental units = 16.

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.

		F344 rats		BN rats	
		p < / PE		p < / PE	
		MAP dark	MAP light	MAP dark	MAP light
		HR dark	HR light	HR dark	HR light
Day 2	Diet board/ Control	NS	NS	0.01 / 1.87	0.01 / 1.89
		NS	NS	NS	NS
	Plain board/ Control	NS	NS	0.001 / 2.25	0.01 / 2.25
		NS	NS	NS	NS
Day 10	Diet board/ Tube	NS	NS	0.01 / 1.91	NS
		NS	NS	NS	NS
	Diet board/ Control	NS	NS	0.01 / 1.91	NS
		NS	NS	NS	NS
Day 14	Tube/ Control	NS	NS	NS	NS
		NS	0.01 / 1.56	NS	NS

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 intervals of a few days. 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 tube group LA was higher than in board groups, and the control group LA was higher than in the plain board group (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.*, 2010), but in both cases, LA differences between groups had disappeared by day 14.

In the study of Kemppinen *et al.* (2010) 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, F344 rats housed with the tube exhibited higher values of MAP compared to both board groups throughout the two-week period. In the conventional cages (as

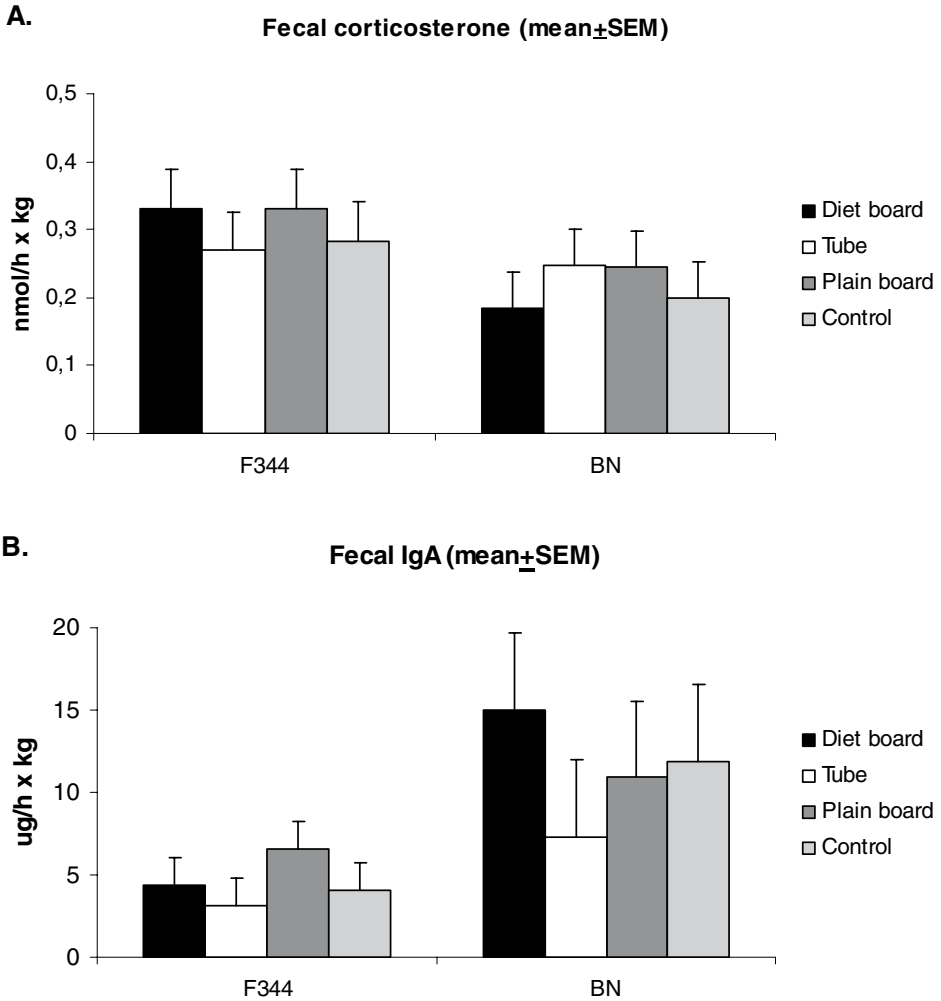


Figure 4. Corticosterone (A) and Immunoglobulin A (IgA, B) excreted via feces in F344 and BN rats with three cage items and control rats. Values expressed as cage means ± SEM nmol corticosterone or µg IgA excreted per hour per kg body weight. Number of experimental units = 16. The F344 rats displayed significantly ($p = 0.05$) higher fecal corticosterone levels than BN rats, while the opposite was true for fecal IgA levels ($p < 0.05$).

used in the present study), the same was observed only on day 14 (Figure 2a). A major difference between conventional cages and IVCs was observed in the BN rats; rats in the IVCs displayed small, 1-2 mmHg, MAP differences between the groups, while

in the conventional 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

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

	F344		BN	
	MAP	HR	MAP	HR
Day 2				
Dark	ns	OPEN ↑ ***	ns	OPEN ↑ ***
Light	OPEN ↑ ***	OPEN ↑ ***	ns	OPEN ↑ ***
Day 6				
Dark	ns	OPEN ↑ ***	ns	OPEN ↑ ***
Light	ns	OPEN ↑ **	ns	OPEN ↑ **
Day 10				
Dark	ns	OPEN ↑ ***	ns	OPEN ↑ ***
Light	ns	OPEN ↑ ***	ns	OPEN ↑ ***
Day 14				
Dark	ns	OPEN ↑ **	ns	OPEN ↑ *
Light	OPEN ↑ **	OPEN ↑ ***	ns	ns

ns = not significance

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., 2010*). 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 rats seemed larger in open cages than in IVCs, whereas in F344 rats CVs appeared to be about the same amplitude (*Kemppinen et al., 2010*).

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 (*Vöipio 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 throughout the present 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 of both strains. This may be due to the fact that the old Wistar rats were two years old, in contrast to the 8-10-month 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 also 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 of rodents. The present study shows that 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 albino F344 rats. 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 differences between the furniture groups to the corresponding strain-specific night-day differences of the controls. We suggest that when between-group differences are smaller than the night-day differences, a result displaying statistical significance is not biologically important (*Kemppinen et al., 2010; 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 and F344 rats in IVCs, both boards were superior to the tube because they lowered the MAP (*Kemppinen et al., 2010*). 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. Contrary to F344 rats, BN rats displayed no biologically significant changes in MAP even though all values were below the control values (Figure 2c).

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 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.

In CV of HR and MAP for 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 presence of 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., 2010*), and this may be due to the novelty effect of the item introduced into the cage on the previous day. The point estimates for HR and MAP in the BN rats were 1.87 - 2.25, and throughout the study they were higher in the open top cages than in the IVCs (*Kemppinen et al., 2010*) and they were higher compared with the F344 rats as well (Table 1). It seems that the BN rats have larger variation for HR and MAP 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.*, 2010), 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 the 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; Pihl & 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 enriched cages compared to the non-enriched cages. However, comparisons with 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 a better representation of the rat as a species (Festing, 2002), and results show that the strains behaved quite differently. Richter *et al.* (2009) argued that genetic and environmental variations cause the poor reproducibility of experimental outcomes. We believe that the wider the range of genetic backgrounds and environments used in a study, the greater the applicability of the results; this is exactly why we used two strains of rats.

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 values 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 board types were superior to the tube. Cage furniture may result in increased variation in the physiological parameters studied and may thus increase the number of animals needed in blood pressure studies, albeit a lack of consistency in the results was 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.

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