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Effects of cage type and gnawing blocks on weight gain, organ weights and open-field behaviour in wistar rats

by *Satu Eskola & Eila Kaliste-Korhonen*, National Laboratory Animal Center,
University of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland.
Correspondence: Satu Eskola, National Laboratory Animal Center,
University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio.
Tel. +358 17 163 346, Fax. +358 17 163 353. E-mail. Satu.Eskola@uku.fi

Introduction

In recent years, the welfare of laboratory animals housed in standard cage types has been criticised and possibilities to improve their living environment have been widely sought. It is recommended that the effects of cage environment on animal welfare should be studied by means of both physiological and behavioural measurements (*Rushen & de Passillé*, 1992). Well growing but unfat animals are in general thought to have good welfare. Stress, on the other hand, has been reported to decrease the body weight, for example in hamsters (*Ibuka et al.*, 1993) and in mice (*Tuli et al.*, 1995). Moreover, the weights of different organs known to react to stress may be used as indicators of welfare. Decrease in the sizes of spleen and thymus as well as enlargement of adrenal glands have been shown to occur in experimental stress procedures (*Hara et al.*, 1981, *Blanchard et al.*, 1993). Furthermore, serum corticosterone concentrations are known to increase during stress (*Blanchard et al.*, 1993, *Tuli et al.*, 1995). The body fat content has been shown to be a useful indicator of physiological burdens (*Bergmann et al.*, 1994/95) and the epididymal adipose tissue weights are appropriate to determine body fat (*Webb & Rogers*, 1979). The brown adipose tissue (BAT) weight is also a part of body fat content. Its hypertrophy or atrophy is induced by cold or heat load of environment as well as overeating or food restriction, which both are associated with the sympathetic nervous system stimulation (*Himms-Hagen*, 1990).

When the effects of environmental changes on behaviour were monitored, open field activity is often used as a test method (*Manosevitz & Pryor*, 1975; *Gentsch et al.*, 1981; *Igarashi & Takeshita*, 1995). In order to compare new results to older ones, the monitoring methods in both studies should be the same. Increased defecation and decreased ambulation in certain parts of open-field arena have been considered as emotional responses or index of timidity (*Archer*, 1973; *Manosevitz & Pryor*, 1975; *Walsh & Cummins*, 1976; *Igarashi & Takeshita*, 1995). On the other hand, ambulation can also be an indication of "active escape" or explorative behaviour (*Archer*, 1973; *Igarashi & Takeshita*, 1995).

The best enrichment object for laboratory rodents is considered to be the one which enhances the normal behaviour of animals (*e.g.* gnawing), is easily kept clean and does not increase the work of animal technicians, is safe and provides stimulation for the animals so that they want to use it (*Chamove*, 1989; *Orok-Edem & Key*, 1994; *Chmiel & Noonan*, 1996; *Renner et al.*, 1992). When rats were offered different kinds of enriching objects, they spent more time with chewable ones (*Chmiel & Noonan*, 1996). In the present study, the aspen gnawing blocks were chosen as enrichment objects because they fulfilled these qualifications.

The aim of this study was to evaluate the long-term effects of cage type and wooden gnawing blocks on the physiology and behaviour of rats. According to our earlier results animals also used them (*Kaliste-Korhonen et al.*, 1995). Furthermore, solid bottom cages with bedding should be better living environment for rats according to the preference tests (*Manser et al.*, 1995; *Manser et al.*, 1996; *Blom et al.*, 1996). Theoretically, a better environment should

increase animal welfare, which should have effects on stress-reactive organs and produce less timid behaviour in open-field. Based on the facts and studies mentioned above, weight gain of the animals, weights of different organs as well as serum corticosterone concentrations - the dominant glucocorticoid in rats (*Evans*, 1996) - were measured to evaluate the effects on their physiology. The behaviour of the animals was tested in a five minute open-field test to assess the effects on behaviour. The analysis of open-field behaviour was divided into two parts (first 2,5 min and second 2,5 min), since it has been stated that the temporal dynamics of motor activity would be more desirable to record than just the total time (*Markel & Galaktionov*, 1989). Furthermore, we re-tested the animals after 4 weeks to investigate, if the enrichment object or caging type would alter the open-field behaviour after a certain period.

Materials and methods

Animals and environment

Male outbred SPF (n=90) Wistar (WH, Hannover origin) rats (National Laboratory Animal Center, Kuopio, Finland) were used in two experiments. Before weaning and before taking the animals into the experiment, they were housed in solid bottom cages with aspen chip bedding (4HP,Tapvei Oy, Kaavi, Finland). At weaning, animals from different litters were mixed after which they were randomly allocated into the groups. The bedding trays under the grid floor cages and solid bottom cages with bedding were changed twice a week, but the grid floor cages them selves were changed once a week. During the experiments, animals were housed in one animal room without contacts to other animals. The ambient temperature of the animal room was maintained at 21 ± 1 °C and the relative humidity at 58 ± 5 %. Light/dark cycle of the animal room was 12:12 hours with lights on at 7.00. Pelleted rat and mouse food (R36, Lactamin AB, Stockholm, Sweden) and tap water were available *ad libitum*. New water bottles, fresh water and new food pellets were given twice a week at the same time with cage or bedding tray changes. Aspen (Populus tremula) gnawing blocks (1x1x5 cm, 2-5 g, Tapvei Oy, Kaavi, Finland) were used as enrichment objects.

Experimental procedures

The first experiment consisted of 54 male rats. Animals were randomised at weaning (at 4 weeks of age) into six test groups; solid bottom-group with (n=9) or without gnawing blocks (n=9), grid floor-group with (n=9) or without gnawing blocks (n=9) and transfer-group with (n=9) or without gnawing blocks (n=9). The rats were housed in groups of three per cage either in stainless steel solid bottom cages (48x28x20 cm) containing aspen bedding (SBC) or in grid floor cages (45x38x19.5 cm, rod diameter 1.6 mm and mesh size 10x10 mm) without contact to bedding (GFC). The transfer-group animals were first housed in SBCs and transferred into GFCs at the age of 8 weeks. The cages in SBC- and transfer-groups were allocated into a cage rack of 4x5 cages so that one cage of each group, with and without blocks, was placed on the three upper levels. The GFCs were in racks of 2x5 cages, one cage with blocks and one cage without blocks on three upper levels. This way it was ensured that each group was represented in each rack level. The light intensities and relative humidity inside the different cages were not measured. Rats in GFC-groups (n=18) and in SBC-groups (n=18) were euthanised at the age of 8 weeks. The animals in transfer-groups (n=18) were first housed in SBCs and transferred into GFCs at the age of 8 weeks and euthanised at the age of 12 weeks.

The experiment was repeated with 36 male rats to investigate more closely the effects of transfer, which were partly missed in the first experiment. The randomisation and housing of animals was conducted similarly as above; SBC-group with (n=9) or without gnawing blocks (n=9) and transfer-group with (n=9) or without gnawing blocks (n=9). The animals in transfer-group (n=18) were first housed in SBCs and transferred into GFCs at the age of 8 weeks. All animals were euthanized at the age of 11 weeks.

The procedures used in this study were in accordance with the European Convention for the

protection of vertebrate animals used for experimental and other scientific purposes (European Convention 1990). The study was approved by the Animal Care and Use Committee of the University of Kuopio.

Use of blocks

Half of the animals in each housing type were provided with pre-weighed, room temperature stored aspen gnawing blocks, three per cage. Animals had one week to habituate to the presence of blocks in their cages before the actual follow up study (starting from the age of 5 weeks). The unused blocks and the remains of blocks fallen through grids in GFCs were replaced with new ones once a week on Mondays. New blocks were provided during the week if needed to ensure that there were always three blocks in the cage. In GFCs new blocks had to be added several times during the week. The collected blocks were dried at room temperature for 24 hours before weighing. The weight loss of blocks was measured per cage (*i.e.* weight losses of three blocks were summarised) and used as an indicator of their use. In the first experiment, the weekly weight losses of blocks during weeks five and six are presented together (but still g/week), because the first actual block change was unfortunately missed. The weight loss of blocks was not recorded when animals were at 8 weeks of age, because the daily and gnawing behaviours of the animals were expected to be disturbed by the presence of researcher during the open field testing and sudden decrease in the number of animals in the housing room due to the euthanization of SBC- and GFC-groups.

Physiological measurements

The growth of the animals was followed by weighing the animals once a week on Wednesdays. Animals were euthanized in a separate necropsy room with CO_2:O_2 -anaesthesia. All the animals to be euthanized during that day were brought to the necropsy room at the same time and euthanized in random order. In the first experiment, the blood was withdrawn from the anaesthetised animals by cardiac puncture between 10:00 - 14:30 hours in two successive days (SBC-groups during the first day and GFC-groups during the second day) or between 8:00 - 11:30 hours (transfer-groups). By placing the animal into an euthanasia chamber with about 70 % CO_2 concentration, the rapid loss of consciousness was reached (European Commission 1995) after which under a constant CO_2:O_2-flow the unconsciousness was maintained and blood was withdrawn within two min. The death of the animal was ensured by cervical dislocation. The serum was separated by centrifuging at 2000 x g for 10 minutes. The sera were frozen at -70 °C until analysis of corticosterone concentrations (n=30 animals) (Radio immunoassay kit, ICN Bio-chemicals, Costa Mesa, CA). The final body weights as well as the weights of adrenal glands, thymus, spleen, interscapular brown adipose tissue and epididymal adipose tissue were measured. The organs were cleaned from the surrounding fat tissue before weighing.

Behavioural measurements

The behaviour test (open-field test) was performed only for the animals in the first experiment. At the age of 8 weeks, the behaviour of all animals was tested in a five min open-field test. The animals transferred into GFCs were re-tested at the age of 12 weeks. The tests were conducted in the same room that housed the animals. The tests were run between 11:00 and 15:00 hours on two successive days (first testing) or during one day (second testing). The animals were tested in random order in a manner that all groups were represented during both test days, and at least one of the animals in each cage was tested at different day than the others. The open-field arena was white and circular, with a diameter of one metre. It was encircled by a 50 cm high grey wall. No extra illumination other than the normal room light (100 - 160 lux one metre from the floor) was used. The animals were placed in the centre of the arena and their behaviour was video recorded. The open-field arena was wiped with mild detergent (Hytox-21, Leverindus, Turku, Finland) after each animal. The behaviour of animals was analysed with a computer-based system (*Jaatinen et al.*, 1989). The behaviour of animals was monitored at the periphery (about 20 cm wide area next to the

wall) and at the central (about 60 cm wide area in the middle) areas of the arena. The behavioural parameters monitored were walking, standing alert (=active but no walking; head movements, slight body movements), rearing (=standing on hind feet with front feet in the air or resting on the wall of open-field arena), grooming (=scratching and pawing them selves with feet or licking the body and feet) and defecation (=boli were detected in the arena). The total frequency and duration, as well as the latency to the first onset of any behaviour were determined from the video recordings. The behaviour of animals in the open-field test was monitored from the video tapes separately during the first (A) and last (B) 2,5 minutes. The walking and rearing behaviours were combined in the statistical tests, since both activities were considered to measure locomotion. The behaviour of animals was not recorded in the home cage, because we could not enable the video recording of GFCs without adding new and different cage lid or contact with floor or bedding tray surfaces during recordings.

Statistical analysis

The data were processed by the SPSS/PC+ V5.1 program (SPSS Europe B.V., Gorinchem, The Netherlands). The distribution of the data was tested with Kolmogorov-Smirnov test. The effects of cage type and presence of gnawing blocks and their interactions were analysed with two-way analysis of variance and the separate differences between groups were further tested with t-test (normally distributed data), Mann-Whitney U-test (non-parametric data) or with manova repeated measures and Friedman-test (repeated measure; parametric or non-parametric data) when necessary. The statistical tests used are indicated in the results. The results are expressed as means±SD. The weight losses of blocks were monitored by cages (n=3 cages per group). The organ weights were adjusted to body weight by using the body weight as the covariate in the statistical tests. The weight gain was calculated from the difference of final body weight and initial body weight.

Results

Use of blocks

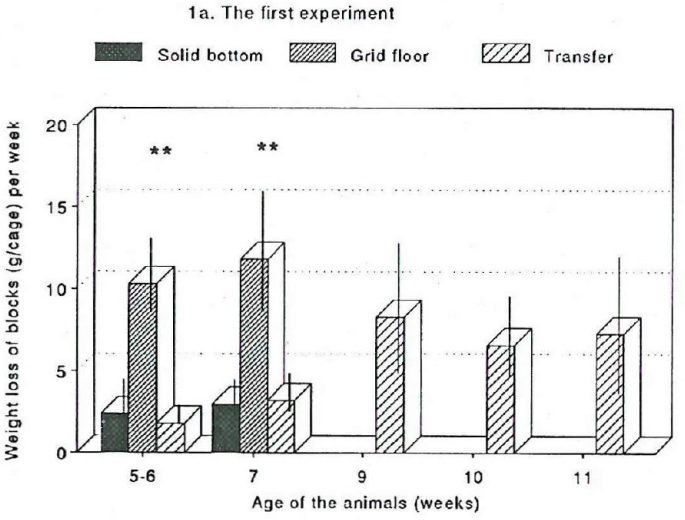
The weight loss of gnawing blocks was used as an indicator of their use. Blocks were clearly used by rats, *i.e.* they were gnawed. The gnawing consisted of chopping the blocks into small pieces. The weekly weight losses of aspen gnawing blocks in the two different cage types are shown in Figure 1. In the first experiment, the weight loss of blocks was about fourfold in GFCs 1-3 weeks after the weaning (Fig 1a). When the rats first housed in SBCs were transferred into GFCs at the age of 8 weeks, the gnawing of the blocks doubled. This was not, however, statistically significant ($p>0.05$ Paired t-test). In the second experiment, the gnawing behaviour of transfer-group increased fourfold after the transfer into GFCs ($p<0.05$ Paired t-test) but the gnawing in SBCs remained similar ($p>0.05$ Manova repeated measures, Fig 1b). The weight losses of blocks in GFCs were threefold higher than in SBCs and the amount of wood gnawed remained at a constant level until the end of the study (Fig 1b). Since the home cage behaviour was not video recorded, it is impossible to say whether all or only some of the animals gnawed the blocks.

Physiological measurements

The statistical analysis for the physiological parameters measured in the first experiment (Table 1), are made without the transfer-group, because of the large difference in age and missing control group.

Effects of blocks: In the first experiment, the animals with gnawing blocks had lower final body weights and lower total weight gains until the age of 8 weeks than animals without blocks (Table 1). The effect of blocks was similar in both cage types (interactions; $p>0.05$). This effect of blocks was not seen in the second experiment (the final body weights on average 297 ± 21 g with blocks vs. 308 ± 27 g without blocks and the total weight gains on average 237 ± 18 g vs. 249 ± 24 g, respectively,

Fig 1. The mean ± SD of weekly weight loss of wooden gnawing blocks (n=3 cages per group; summarized weight losses of 3 blocks per cage) in solid bottom cages and in grid floor cages. Transfer = animals were transferred from SBC into GFC at the age of 8 weeks. 1a = The first experiment, 1b = The second experiment.
** The effect of cage type $p<0.01$ (Manova repeated measures).
a The effect of transfer $p<0.05$ (Paired t-test).



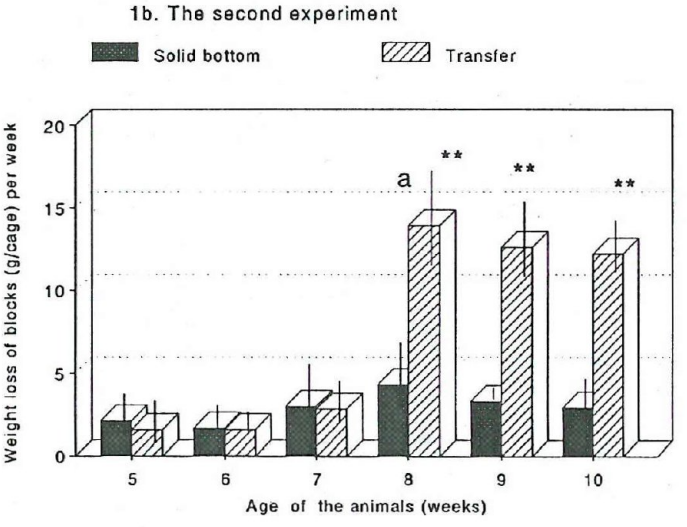


Table 1. Mean values ± SD and p-values (Analysis of Variance) by cage type, availability of blocks and their interaction for physiological parameters measured at the end of the first experiment (animals housed in SBCs or in GFCs from weaning) and second experiment during which, half of the animals were housed in SBCs during the whole study and the other half was transferred from SBCs at the age of 8 weeks into GFCs for 3 weeks, (n=9 animals / group). a = In the Analysis of Variance, the final body weight was taken as covariate, the covariate had a significant effect on adrenals p=0.000 and BAT p=0.013.

	Solid bottom cages		Grid floor cages		P-values		
	Blocks	**No blocks**	**Blocks**	**No blocks**	**Cage type F(df)**	**Blocks F(df)**	**Interaction F(df)**
First experiment at the age of 8 weeks							
Final body weight (g)	222±18	230±15	237±16	257±20	**0.001** 13.2(1,32)	**0.030** 5.2(1,32	0.319 1.0(1,32
Weight gain (g)	113±5	123±11	125±9	135±5	**0.000** 19.0(1,32)	**0.001** 13.4(1,32)	0.928 0.01(1,32)
Adrenals (mg) [a]	55±6	61±6	70±8	72±9	**0.001** 12.3(1,31)	0.422 0.7(1,31)	0.147 2.2(1,31)
Serum corticosterone concentration (ng/ml), (n=5 / group)	281±107	279±169	105±124	77±66	**0.003** 11.9(1,16)	0.789 0.1(1,16)	0.804 0.1(1,16)
Second experiment at the age of 11 weeks							
Adrenals (mg) [a]	66±9	75±14	74±9	76±8	**0.046** 4.3(1,31)	0.394 0.7(1,31)	0.511 0.4(1,31)
Brown adipose tissue (mg) [a]	549±138	513±84	521±73	663±144	**0.051** 4.1(1,31)	0.392 0.8(1,31)	**0.006** 8.9(1,31)

$p>0.05$ Analysis of Variance). Otherwise, the adrenal glands and serum corticosterone concentrations (Table 1) or the spleen (overall mean: first experiment 739 ± 97 mg and second experiment 794 + 126 mg), thymus (862 ± 102 mg and 883 ± 170 mg) and epididymal adipose tissue weights (2.7 ± 0.6 g and 4.9 ± 1.4 g, respectively) were not significantly affected by the presence of gnawing blocks. Moreover, serum corticosterone concentrations were not correlated with adrenal weights (Pearson coefficient -0.439, $p>0.05$).

Effects of cage type: In the first experiment animals housed in SBCs had lower final body weights and smaller total weight gains until the age of 8 weeks than animals housed in GFCs (Table 1), even though there were no differences in initial body weights or in separate weekly body weights. The effect of cage type on final body weights and weight gains was not found in the second experiment (the final body weights on average 305 ± 28 g in SBCs vs. 299 ± 20 g in GFCs, the weight gains at the age of 8 weeks 154 ± 15 vs. 149 ± 14 g and at the age of 11 weeks 246 ± 25 g vs. 241 ± 19 g, respectively, $p>0.05$ Analysis of Variance). The animals housed in GFCs in both experiments had larger adrenal glands than animals housed in SBCs, but lower serum corticosterone concentrations in the first experiment (Table 1). In the second experiment, the weights of brown adipose tissues had a tendency to be larger in animals housed in GFCs (Table 1). The significant interaction of cage type and blocks shows that the weight difference between animals with blocks and animals without blocks in SBCs is opposite to that of weights in GFCs (Table 1). Otherwise, the spleen, thymus or epididymal adipose tissue weights were not affected by cage type (overall means mentioned in the paragraph Effects of blocks).

Behavioural measurements

The analysis of open-field behaviour was divided into two separate parts (A=first 2,5 min and B=second 2,5 min), which both equalled 100 %, *i.e.* periphery A and central A behaviours together represent 150 seconds. The rats housed in GFCs from weaning groomed significantly less in the periphery during the last period of the test than animals housed in SBCs (Table 2). Grooming behaviour was totally absent in the central area in all test groups. There were minor differences in the locomotion activity in the central area: rats without gnawing blocks in both cage types decreased their activity in the central area of the arena during the last 2,5 minutes of the test (Figure 2a). In GFCs, this decrease of activity was great enough to produce a significant difference between the groups with or without blocks (Fig 2a). The locomotion activity in the peripheral area (Figure 2a) and the standing alert behaviour (Table 2) were not affected by the cage type or presence of blocks in the first open-field test. Moreover, the defecation frequencies during the first test were similar in all six groups (overall means; 1.2 +1.6 first 2,5 min and 0.8 ± 1 second 2,5 min)

After transferred into GFCs, the grooming behaviour of the animals decreased (Table 2). The locomotion activity in the central area during the first 2,5 minutes decreased in animals with blocks (Figure 2b) and during the second 2,5 minutes in animals without blocks (Figure 2b), when re-tested at the age of 12 weeks. The animals in GFCs without gnawing blocks were less active in the peripheral area during the first half of the test than animals with blocks (Figure 2b) and their standing alert behaviour was correspondingly increased (Table 2). The defecation frequencies were not affected by the presence of blocks or the cage type (0.8 ± 1.2 first 2,5 min and 0.2 ± 0.4 second 2,5 min).

Discussion

The weight losses of blocks were about four fold in GFCs when compared to the ones in SBCs. Furthermore, gnawing of the blocks remained at constant level throughout the studies. This suggests that the animals in GFCs really used the blocks and the blocks maintained their attractiveness, *i.e.* animals did not get bored with them over time. Previously it has been shown that in SBCs rats spent only few minutes with the blocks during 24 hours and gnawing was the most active and long-lasting activity with them (*Kaliste-Korhonen et al.*, 1995), thus the gnawing was chosen as an indictor of the block usage. In

Table 2.
Effects of cage type and presence of gnawing blocks on behaviour in the 5 min open-field test for Solid bottom cage- and Grid floor cage-groups at the age of 8 weeks and for Transfer-group before transfer (at the age of 8 weeks) and after transfer to grid floor cages (at the age of 12 weeks). A= first 2,5 min, B= second 2,5 min, (means ± SD, n=9 animals/group).

Behaviour parameters	Solid bottom cage		Grid floor cage		Before transfer		After transfer	
	Blocks	No blocks	Blocks	No blocks	Blocks	No blocks	Blocks	No blocks
GROOMING (% of time)								
Periphery A	1±2	2±3	0.5±1	0.2±0.4	2±2	2±3 *	0.1±0.3	0±0
Periphery B	2±3	4±3 ***	0.3±1	0.1±0.4	3±3	4±5 *	1.6±2.4	0.4±0.7
STANDING ALERT (% OF TIME)								
Central A	3±2	3±2	3±3	6±5	4±3	4±5	2±3	4±10
Central B	1±1	1±1	2±2	0.2±0.6	2±4	0.4±0.7	1±2	0±0
Periphery A	29±14	23±16	23±8	20±5	21±13	30±21	30±9 **	51±18
Periphery B	22±9	25±7	24±9	30±13	20±5	33±23	34±11	55±27

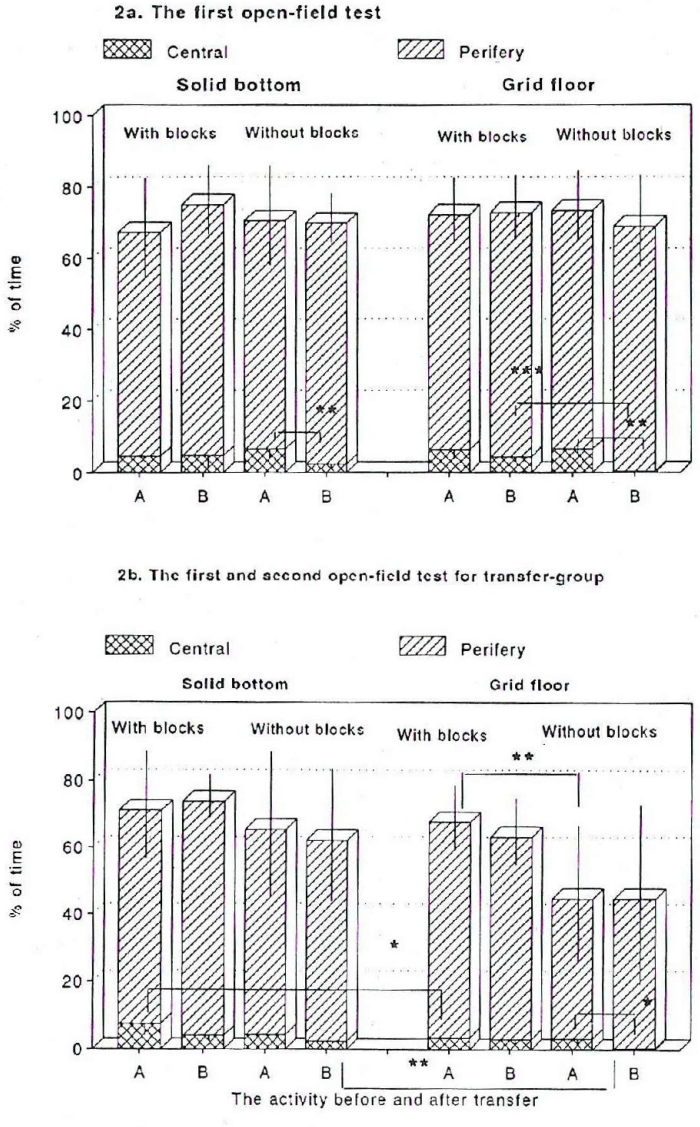
***, $p<0.001$ (Mann-Whitney U-test), the effect of cage type. **, $p<0.01$ (Mann-Whitney U-test), the effect of blocks. *, $p<0.05$ (Friedman), the effect of cage type.

Fig 2. The mean ± SD percentages of locomotion activity in central and periphery area during 5 min open-field test. 2a = The animals at the age of 8 weeks, 2b = The animals tested first at the age of 8 weeks, transferred into GFCs and tested at the age of 12 weeks. A = first 2,5 min, B = second 2,5 min. n=9 animals / group.

* $p<0.05$, Friedman test.

** $p<0.01$, Friedman or Mann-Whitney U-test.

*** $p<0.001$, Mann-Whitney U-test.



this study, the weight loss of blocks in SBCs was quite minimal when compared to the weight loss in GFCs, which is in accordance with the earlier results (*Kaliste-Korhonen et al.*, 1995) When the animals were transferred from SBCs into GFCs, the gnawing of the blocks doubled. Hence, the blocks may have a more enriching value for rats in GFCs than in SBCs.

Effect of gnawing blocks

In open-field tests, the animals without blocks in both cage types decreased their activity in the central area during the last 2,5 minutes more clearly than animals with blocks. Avoidance of the central, open area in the open field test is thought to be a sign of emotionality or fear (*Ossenkopp et al.*, 1994). Moreover, animals transferred to GFCs at the age of 8 weeks without blocks showed decreased locomotion activity in the peripheral area after the transfer. The activity of rats in GFCs with blocks instead resembled the behaviour of animals in SBCs. When animals are tested more than once, a habituation for the test situation may have effects on the behaviour. However, in this study the time period between the tests was long, and the habituation effect should be similar in both groups. Since the behaviour changes in groups with and without blocks were not similar, the general habituation effect on the behaviour can be excluded. Accordingly, the possibility to use blocks seemed to make the rats less emotional and more active. However, defecation, the other parameter claimed to indicate emotionality (*Archer*, 1973; *Ossenkopp et al.*, 1994), was not influenced by the presence of blocks. Furthermore, the differences between groups were so small, that no strong conclusions can be drawn.

In the first experiment, the animals with gnawing blocks had less weight gain than the animals without blocks. This might be due to the manipulation of food as play object in the absence of gnawing blocks and/or bedding, thus resulting in greater food intake and weight gain. Eating of blocks could also reduce the food intake and cause decrease in the weight gain. However, there was no evidence of wood particles in the stomach during the autopsy (but food particles were present) and the chopped blocks were detected inside the SBCs or on the bedding trays under the GFCs. Since the food intake was not monitored nor the home cage behaviour, we cannot determine if the increased food consumption was actually the case in this study. However, it has been shown that "impoverished" rats weigh more than "enriched" rats, because they eat more (*Fiala et al.*, 1977). The other physiological variables measured in this study were not influenced by the presence of blocks and these effects could not be detected in the second experiment either. This indicates that the aspen blocks would not have harmful effects on experimental results.

According to Cubbitt (1992) and Chmiel & Noonan (1996), enrichment objects provided to experimental animals should be safe, economical, easily cleaned and suitable for the enrichment purpose. Aspen gnawing blocks fulfil most of these qualities: they are cheap and easily manufactured, and they can be easily replaced with new blocks during cage changing. Moreover, rats clearly used them in GFCs, *i.e.* blocks may encourage the normal gnawing behaviour of rodents. If made from the same material as the bedding, they do not introduce any extra chemical compounds into the cage environment. However, hardwood and softwood materials have been shown to have some cytotoxic properties (*Potgieter et al.*, 1995; *Pelkonen & Hänninen*, 1997). The usage of wood blocks in toxicity tests, if not made from the same material as bedding, should be carefully considered. In conclusion, aspen gnawing blocks may be recommended for enrichment, especially in GFCs.

Effect of cage type

The cage type affected the grooming behaviour and locomotion activity in the open field; rats housed in GFCs groomed less in both tests and were less active in the second test than rats from SBCs. Grooming behaviour has been shown to indicate adaptation to the test situation (*File et al.*, 1988) and greater locomotion in the peripheral areas rather than in inner areas of open-field to be an indication of timidity (*Walsh & Cummins*, 1976). Living in GFCs might not provide enough stimulation for rats, leading to more passive and emotional behaviour in a novel environment.

Animals housed in SBCs seemed to be less timid and they probably had better ability to cope with the novel situation as suggested by the minor increase in grooming behaviour in SBC rats during the last 2,5 minutes. The defecation frequencies, however, were not influenced by the cage type.

The cage type had greater influence on the physiology of rats than the presence of gnawing blocks. The greater weight gain in GFCs might be due to a smaller energy consumption or greater food intake. The bedding material offers the animals something to manipulate, probably resulting in more active animals in SBCs and hence greater energy consumption. The decreasing effect of blocks on weight gain supports this hypothesis; blocks probably also activated the animals. The rats without bedding and blocks in GFCs, on the other hand, had only food as material with which to interact and the animals might have eaten more as a result leading this way to more fat animals.

The greater weight gain in GFCs might also be due to a lower temperature in GFCs than in SBCs. The greater weight gains and the tendencies of brown adipose tissue weights to be higher in GFCs may indicate physiological adaptation to colder environment (*Himms-Hagen,* 1990). However, the epididymal adipose tissue weights were not influenced by the cage type, as could have been expected according the cold acclimation hypothesis in which the amount of fat would accumulate in colder environment. All cages were in the same animal room so the macro environment was similar to all animals, but the micro environment may vary in SBCs and in GFCs due to the presence / absence of protective walls and insulating effect of bedding. The temperature inside the cages was not measured in this study, but later measurements in similar conditions have shown that the temperature is about 0,5 °C higher inside the GFCs than in SBCs. According to these details, the cold acclimation hypothesis does not seem probable.

The size of adrenal glands and concentration of serum glucocorticoids are often considered to be stress indicators. Chronic stress has often been shown to enlarge the adrenal glands (*Kvetňanský & Mikulaj,* 1970; *Hara et al.,* 1981) and increase circulating ACTH leading to higher glucocorticoid secretion (*Stratakis & Chrousos,* 1995). There are, however, large differences between strains in responses of serum ACTH, corticosterone and adrenal weights to chronic stress in inbred rat (*Gòmez et al.,* 1996); some of the strains tested did not react to chronic immobilization stress with adrenal hypertrophy or increased levels of serum hormones. Moreover, a negative correlation between adrenal weight and serum corticosterone concentration has been found after some stress treatments (*Gòmez et al.,* 1996), of which was also an indication in our study. These findings emphazise that the welfare or stress of animals should not be evaluated by using only a single parameter (*Rushen & de Passillé,* 1992). In this study, the more activating environment in SBCs may lead to increased activity of the hypothalamus-pituitary-adrenal axis and thus explain the higher concentration of serum corticosterones in SBCs. On the other hand, a more simple explanation for this difference might be the euthanization procedure; the animals in SBCs were euthanized during one day and the GFC-animals during the following day. The other stress parameters (weights of adrenals, thymus, spleen) were not in accordance with the levels of serum corticosterone, indicating that the increased corticosterone levels in this study may not be a sign of stress.

Overall, SBCs with bedding decreased weight gain and adrenal size in rats, but increased the serum corticosterone concentration. The rearing environment slightly affected the open-field behaviour. The main factor underlying the differences between cage types is probably the presence of bedding. The results suggest the importance of bedding as an environmental factor for rats. SBCs with bedding are in fact recommended for housing of laboratory rats (*Weihe,* 1987; *Manser et al.,* 1995; *Roe et al.,* 1995; *Manser et al.,* 1996). On the other hand, *Nagel & Stauffacher* (1994) have stated that there were no differences in resting and exploration behaviours or adrenal weights and corticosterone concentrations in rats housed in full wire cages when compared to animals in solid bottom cages with bedding.

In summary, the aspen blocks were significantly gnawed in GFCs, they slightly decreased the adrenal weights in SBCs and increased open-field activity. The blocks also seemed to decrease the weight gain, resulting less fat animals. Harmful effects of gnawing blocks were not found. In conclusion, the gnawing blocks might have some positive effects on animals and could be recommended as enrichment objects. Housing in SBCs decreased weight gain and brown adipose tissue weight compared to GFC housing. It also increased the serum corticosterone concentrations but decreased the weights of adrenals. The locomotion activity and grooming behaviour of SBC housed animals were increased in the open-field test, which might indicate less timid and more explorative animals. The presence of gnawing blocks seemed to antagonize the effects of housing in GFCs. Since the animals also used the blocks more efficiently in GFCs, they can be recommended as enrichment in this housing type.

Summary

Two separate experiments were conducted to study the environmental enrichment value of aspen gnawing blocks in solid bottom cages with bedding (SBC) and in grid floor cages without bedding (GFC), and the effects of housing environments on the physiology and behaviour of male outbred Wistar rats (n=90). Animals were housed in groups of 3 from weaning until the age of 8-12 weeks. The behaviour of animals in the first experiment was tested in five minute open-field tests at the age of 8 and 12 weeks. Rats gnawed blocks about four times more in GFCs than in SBCs ($p<0.01$). In the first experiment, animals housed in GFCs had heavier adrenal glands ($p<0.001$) but lower serum corticosterone concentrations ($p<0.01$) and their weight gain was greater than animals housed in SBCs ($p<0.000$). The presence of blocks in cages decreased the weight gain in both cage types ($p<0.001$). In the first open-field test, the animals without blocks in both cage types decreased their activity in the central area during the last 2,5 min of the test ($p<0.01$). The similar effect of blocks was also seen in animals later transferred into GFCs ($p<0.05$). These rats without blocks were also less active in the periphery ($p<0.01$) and had more standing alert behaviour ($p<0.01$) than animals with gnawing blocks. In both open-field tests, rats housed in SBCs showed more grooming behaviour than animals in GFCs ($p<0.05$). In the second experiment, animals in GFCs had again enlarged adrenals ($p<0.05$) and their brown adipose tissue weights were slightly increased ($p<0.05$). Altogether, SBC as a living environment resulted in lighter animals with smaller adrenals, but higher serum corticosterone concentrations. In the open-field, blocks seemed to result in more active and less timid animals and antagonize the effects of housing in GFCs. Aspen gnawing blocks can be recommended as enrichment objects especially in GFCs.

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