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Effects of gnawing material, group size and cage level in rack on Wistar rats

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Introduction

All animals are products of their environment. Until now, the complexicity and variation of the laboratory animals have been regarded disadvantageous, hence their environment has been standardized as much as possible. Even in such an environment the living conditions of rodents can vary remarkably depending on, for example, the number of cagemates, existence and quality of bedding material and lightning conditions. Social relationships are thought to be particularly important for the colonial rats. Individually housed rats are said to suffer from 'isolation stress' or 'social deprivation' (*Brain & Benton* 1979).

The standardization dogma is being replaced with the ideas of environmental enrichment. In recent studies enrichment has been used in order to increase the well-being of rats (*Watson* 1993, *Orok-Edem & Key* 1994), mice (*Watson* 1993) and rabbits (*Brooks et al.* 1993). The methods of enrichment vary ranging from added objects to changes in the social environment. Generally, it is concluded that enrichment is beneficial for the animals if they 'used' the enrichment object. Whether this is valid criteria for the physiological or psychological welfare, has neither been studied nor demonstrated.

Since rodents are supposed to have a need for gnawing, an object fulfilling this need can be expected to have a positive value. This study was designed to asses the enrichment value of wooden blocks for laboratory rats by measuring both the use of the blocks and their effects on the animals. Moreover, the possible effects of group size and level of cage in cage rack were evaluated.

Materials and Methods

Animal and design

Outbred Han:Wist rats (National Laboratory Animal Center, Kuopio, Finland) were used in the study. The ambient temperature of the animal room was maintained at 21 ± 1 °C and the relative humidity at 50 ± 10 %. Light/dark cycle of the animal room was 12:12 hour with lights on at 7.00. The pelleted rat food (R3, Lactamin AB, Stockholm, Sweden) and tap water were available *ad libitum*. The direct or indirect bedding used was aspen chips (Tapvei Oy, Kaavi, Finland). Blocks of aspen (1 × 1 × 5 cm, weight 2–5 g) served as the enrichment objects. They were dried at 60° C for a couple of days prior to introduction into cages.

The design of the experiment is shown in Table 1. The animals (52 males and 26 females) were randomized at weaning into groups of one, two, three or four and housed in stainless steel solid bottom cages (42 × 25 cm, height 15 cm) on contact bedding. The cages were randomized on five shelves, five cages on each. Males and females were kept in separate racks. The cages were changed twice a week. The light intensity in the cages ranged from 200 lux on the highest shelves to 25 lux on the lowest ones. When the rats reached the age of nine weeks, groups with three or four animals in a cage were transferred into larger cages (48 × 28 cm, height 20 cm). At the age of fourteen weeks the females were taken away from the study but the males were transferred into suspended stainless steel cages with wire mesh bottoms and fronts (45 × 38 cm, height 19.5 cm) with bedding trays underneath. The racks for

Table 1. Experimental design.

Age (weeks)	Procedure/Determination
3	Randomization: 1, 2, 3 or 4 animals in cage, 5 shelves Housing in solid bottom cages with bedding Blocks into cages
3–7	Monitoring food intake
4–6	Monitoring block gnawing
7	Monitoring block gnawing in light and dark periods
8	Males: open field test
9	Animals in groups of 3 or 4 into larger solid bottom cages
10–13	24-hour video recordings
12–14	Monitoring block gnawing
14	Males into cages with wire mesh bottom, females eliminated
14–18	Monitoring block gnawing
3–18	Weekly weighing
18	Euthanasia, adrenals, thymus, spleen and body weighing

these cages had five shelves likewise, but only two cages on each shelf. The light intensity in these cages ranged from 10–20 lux on the highest to 5 lux on the lowest shelves. The trays were changed twice weekly but the cages once a week.

Use of the blocks

Half of the males and all females were provided with pre-weighed wooden blocks in their cages; the number of blocks in a cage equalled the number of the animals in that cage. The females were included in the study to find out whether there were sex differences in the use of blocks. The blocks were observed daily and replaced with new ones whenever needed, also during cage changing. The blocks were always dried at 60° C for at least 24 hours prior to weighing. The weight loss of the blocks was used as an indicator of their use. The block-related activity of the animals were monitored by 24 hours video recording.

Physiological measurements

The food consumption for each cage was assessed by measuring the food loss for three days during each of the first four weeks. The growth of the animals was monitored by weekly weighing throughout the study.

At the end of the study, when the animals reached the age of 18 weeks, they were euthanized and their body weights, as well as the weights of the adrenals, thymus and spleen – organs known susceptible to stress – were measured.

Behavioural measurements

When the rats were eight weeks old, behaviour of the males was tested in an open field arena in a separate animal room. The animals were transferred into that room one by one in a transport cage with bedding. The open field arena was white and circular, with a diameter of one meter. It was encircled by a 50 cm high grey wall. The illumination in the centre of the open field was 380 lux. A 80 dB masking noise was provided by a white noise generator. The animals were placed in the center of the arena and their behaviour was monitored with the aid of a video camera for five minutes. The open-field test was run for two days between 9.00 and 13.00 hours. The animals were taken to the test from each test group in turn. The test arena was wipped with mild detergent after each animal.

The animal activity data were processed with a computer-based system (*Sallinen & Hatunen* 1992), where the video signal is transferred to a computer via a video-digitizer. Changes between two subsequent images can be detected by subtracting the second picture from the first one and yielding a numerical value for the animal's movement. This recording method makes it possible to register the animal's presence and movements in different areas of the test arena and at different times selected freely after the video recording. In the present study, the arena was divided into 21 square frames. The numerical values of the activity (frequency of movements) in different frames were further combined so that the final areas to be studied were the central area, about

20 × 20 cm in the center of the arena, the peripheral area consisting of about 20 cm wide area at the periphery, and the intermediate space, about 20 cm wide area between the two others. Ultimately, the central and intermediate areas were combined.

The other behavioural parameters monitored were walking, standard alert (= active but no walking), rearing, grooming and defecation. The total frequency and duration of these variables during the open field test, as well as the latency to the first onset of any behaviour were analysed on the basis of the video taped material.

Data processing

The distribution of the data was tested with Kolmogorov-Smirnov test. The statistical analyses were done using tests indicated in the Results. Data were processed by the SPSS/PC + V3.1 program (SPSS Europe B. V., Gorinchem, The Netherlands). The results are expressed as mean ± standard deviation (SD). The independent factors studied were: age, sex, cage type, group size, level of the cage in the rack (cage level) and the presence of blocks in a cage. Weight losses of blocks and food intake were monitored by cage and further divided by the number of animals. Organ weights were adjusted to body weight by analysis of covariance using the body weight as the covariate. The open field test generates a lot of data with some seemingly occasional differences between the groups. To reduce the number of the behavioural variables they were subjected to a factor analysis with orthogonal VARIMAX rotation and Kaiser normalization. This analysis combines the correlated variables into one and the same factors.

Results

Use of the blocks

The block related activity of rats was monitored with a 24 hour video recording. In solid bottom cages the animals manipulated blocks only occasionally. The total time used with blocks was only a few minutes during the 24 hour follow-up period (Table 2). Most of the contacts included touching the objects with nose or forepaws, sniffing or biting them and carrying them from place to place. Episodes of intensive gnawing over 30 seconds were rare. However, since gnawing was the most active and long-lasting activity with the blocks, it was chosen as an indicator of the block use.

In solid bottom cages with bedding, gnawing was minimal, daily weight loss of the blocks being only 0.2 ± 0.1 g/animal. The group size or cage level in racks did not affect gnawing and neither did age or sex of the animals (data not shown). However, gnawing increased remarkably when the animals were transferred from solid bottom cages

Table 2. Group size and contacts with wooden blocks in solid bottom cages with bedding during the follow-up period of 24 hours. Results are expressed as mean ± SD.

	Group size		
	1	2	3
Total animal number	4	4	3
Contacts:			
Frequency (n/24 h)	12 ± 5	8 ± 4	5 ± 1
Frequencies (n/24 h):			
< 10 s	8 ± 4	6 ± 3	2 ± 1
10–30 s	3 ± 1	2 ± 1	2 ± 1
> 30 s	0.3 ± 0.4	0.5 ± 1	1 ± 1
Total contact time (s)	93 ± 44	84 ± 10	103 ± 37
% in dark period	85 ± 5	78 ± 10	87 ± 18

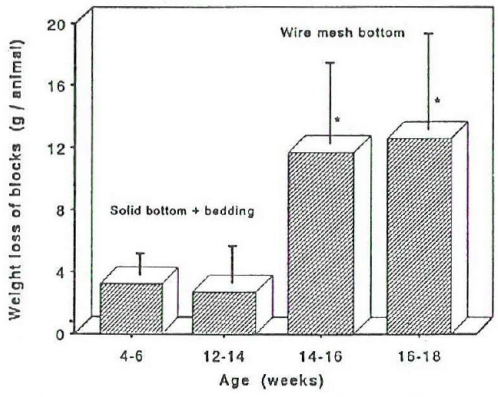

Figure 1. Weight loss of aspen blocks during 2 weeks in male rats housed in different cages. Means ± SDs are shown, n = 26. * $p < 0.001$ when compared to the weight loss in solid bottom cages at the age of 12–14 weeks (paired t-test).

with bedding into cages with wire grid bottom (Figure 1).

The weight loss of the blocks took place mainly during the dark period (Figure 2). The consumption of the blocks was largest on the third shelf of the rack both during the light and dark period. Significant difference was, however, found only between shelves 1 and 3 and only during the light period. Light intensity, as a main factor, did not influence gnawing significantly.

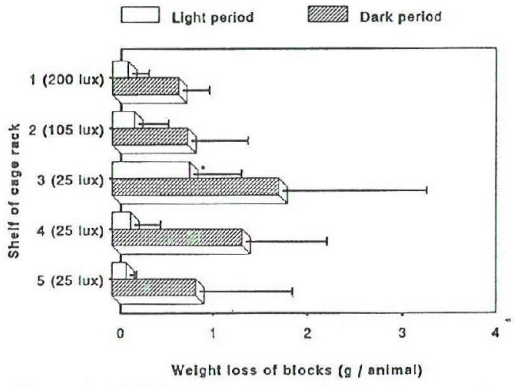

Figure 2. Weight loss of wooden blocks over 4 days during 12 h light and 12 h dark periods. Means ± SDs are shown, n = 26. * $p < 0.05$ between the groups (Scheffe's test).

Physiological measurements

Effects of blocks: In solid bottom cages, the presence of the blocks in a cage did not affect food consumption or the weight gain of the animals (data not shown). The consumption of the blocks increased with the increasing food intake in males (Pearson's coefficient 0.94, $p < 0.01$) but not in females (0.62, not significant). The presence of the blocks in cages with wire mesh bottom was associated with significantly decreased weight of the adrenals: the weight in animals with blocks was 54.0 ± 13.3 mg and in animals without blocks 60.5 ± 11.8 mg ($p = 0.04$, Anova). The other organ weights were not affected by the blocks.

Effects of animal number in cage: The food consumption of the animals or their weight gain was not influenced by the group size. Neither did the group size affect the organ weights of the rats (data not shown).

Effects of cage level: The cage level in rack did not influence any of the physiological parameters measured (data not shown).

Behavioural measurements

The activity recording method used in the study allowed a detailed analysis of the minute-by-minute activity in the central and peripheral areas of the open field. The activity changed over time both in central and peripheral areas ($p = 0.000$ and 0.04, respectively; repeated measures of Manova). The independent variables tested affected only some behaviours in the open field test (Kruskal-Wallis one-way anova). The behaviours with significant differences are presented in Table 3. The differences between the groups were tested by the method of multiple comparison between groups according to *Siegel* (1988). Due to the conservative nature of this test, it revealed only a few differences between the groups despite the results of Anova.

Effects of blocks: The blocks in cage did not affect the activity over time in open field or any other behaviours monitored.

Effects of group size: The group size had a

Table 3. Effect of group size and cage level on some behaviours in the open field arena. Means ± SDs are given, superscript indicates groups from which marked groups differ with $p < 0.05$. (Multiple comparison between groups according to *Siegel* (1988).

The variable	Group size				
	1 (n = 12)	2 (n = 12)	3 (n = 12)	4 (n = 16)	p*
Walking:					
% of time	31 ± 13	40 ± 14[4]	33 ± 7	30 ± 7	0.04
Rearing:					
Latency (s)	21 ± 20	17 ± 7	16 ± 11	8 ± 3[2]	0.01
Frequency	21 ± 18	23 ± 7	31 ± 7	29 ± 9	0.05
% of time	14 ± 11	13 ± 5	19 ± 4	20 ± 5	0.01
	Cage level**				
	1 (n = 9)	2 (n = 13)	3 (n = 16)	4 (n = 10)	5 (n = 4)	p*
Rearing:						
Latency (s)	28 ± 20[2,4]	9 ± 4	15 ± 10	10 ± 6	12 ± 7	0.00
Grooming:						
Latency (s)	115 ± 56	178 ± 86	142 ± 70	189 ± 82	250 ± 35	0.02

* Kruskal-Wallis one-way anova.
** Cage level: 1 = the highest, 5 = the lowest shelf of the rack.

significant effect on the change of the activity over time in the peripheral area ($p = 0.02$) but not in the central area ($p = 0.58$) (Figure 3). The rats living alone in the cage increased their activities in the periphery during the last minute whereas with group housed animals the activity decreased. During the first four minutes, the singly housed rats moved less as compared to the other rats. However, during the fifth minute, there were no more differences between the groups. The animals living in groups of three or four increased their activity in the central area after three minutes, whereas the smaller groups did not.

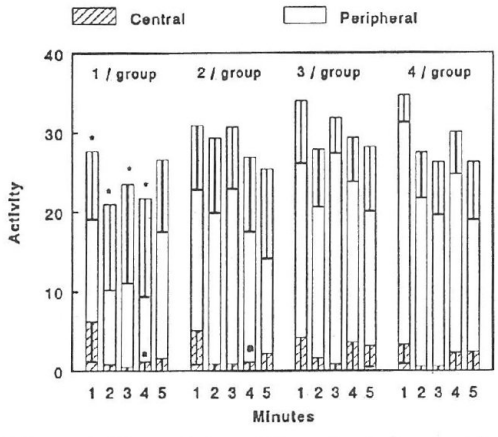

Figure 3. The activity of the animals in central and peripheral areas of open field arena, n = 12–16 ± SD. * $p < 0.05$, peripheral activity differed from the other groups.
[a] $p < 0.03$, central activity differed from the groups of three or four animals (Manova-analysis).

The singly housed rats showed in general more variation in behavioural measurements than the animals with cage mates (Table 3). The animals with one partner were most active, as judged from the time spent walking. Compared to the groups of three or four animals, the number of rearings and the time spent in rearing were both reduced in groups of one or two rats. The latency time of rearing was shortest in groups of four animals.

Effects of cage level: The cage level did not have effects on the activity of the rats in open field. The animals living on the highest shelf of the rack showed a longer latency in rearing and shorter latency times in grooming (Table 3).

Table 4. Varimax rotated factor matrix for the variables in the open field. Loadings smaller than 0.4 are omitted.

Variables	Fac 1	Fac 2	Fac 3	Fac 4	Fac 5	Fac 6
Activity in total area						
1. min	.577			−.456		
2. min	.864					
3. min	.816					
4. min	.837					
5. min	.594	.466			−.393	
Total	.949					
Activity in central area						
1. min				.871		
2. min					.499	
3. min						
4. min					.791	
5. min						
Total				.543	.729	
Activity in peripheral area						
1. min	.443			−.831		
2. min	.880					
3. min	.825					
4. min	.826					
5. min	.521	.450			−.485	
Total	.935					
Behavioural variables						
Walking freq.	.776					
Walking lat.	−.439		−.533			
Walking time	.783					
Rearing freq.	.545		.636			
Rearing lat.		−.461	−.595	.421		
Rearing time	.468		.758			
Grooming freq.		−.892				
Grooming lat.		.564				
Grooming time		−.854				
Standing time	−.795		−.409			
Defecation freq.						−.871
Defecation lat.						.888
% of variance	39	9	8	8	6	5

Factor analysis: The Varimax rotated factor matrix for the behavioural variables is presented in Table 4. Six factors were extracted, altogether accounting for 75 % of the variance. Factor 1 received its highest loadings from the total and peripheral activities as well as from walking, rearing and standing alert. It accounted for 39 % of the variance. The peripheral activity during the last minute and the grooming behaviour loaded on Factor 2. The rearing behaviour, the latency in walking and standing time loaded on Factor 3. The activity during the first minute as well as the total central activity loaded on Factor 4 with the latency in rearing. General central activity, excluding the first minute, loaded on Factor 5. Defecation variables were separately loaded on Factor 6. The group size, cage level or presence of blocks did not affect the factor scores according to Anova analysis (data not shown).

Discussion

The present study assessed utilization of the wooden blocks both with video recording and weight loss of the blocks. In solid bot-

tom cages with bedding, the use of the blocks was limited, consisting of only 5–20 short contacts with the objects over 24 hours and minimal weight loss of the blocks. This indicates a minor enrichment value of the blocks. Similar results in rats have been reported by *Hirsjärvi* (1994). Apparently, wooden blocks did not give any additional enrichment for rats besides the bedding. Large increase in gnawing activity after transferring the animals into grid floor cages without direct bedding supports this conclusion. The gnawing of the blocks in the grid floor cages could be an attempt to provide the contact bedding to which the rats were used.

The presence of blocks in a cage did not change food intake, weight gain or behavioural parameters recorded in this study. It is hardly surprising if one considers the minimal use of the blocks in solid bottom cages. These findings confirm *Watson*'s study (1993) which did not show any changes in food intake, weight gain, hematology or clinical chemistry of rats which could be attributed to gnawing material.

After four weeks of keeping animals on the grid floor, the animals with wooden blocks in their cages had smaller adrenals than the others. This finding may indicate that the blocks decreased the stress of rats caused by the change of their environment, i.e. the removal of bedding. The increase of gnawing after the rats had been transferred into the wire mesh cages suggests also, that the wooden blocks may have served as a substitute for bedding.

Single housing is regarded as a stressful situation with physiological and behavioural consequences such as enlarged adrenals, smaller brain size, increased or decreased adrenocortical activity and increased emotionality (*Brain & Benton* 1979). However, the literature does not conclusively confirm the stressfulness of the single housing (*Brain & Benton* 1979). The possible reasons for the diversity of the results include duration of individual/group housing, age, strain and sex of animals, interaction of experimental procedures with results, and composition of the groups compared with the individual housing (strange or familiar cage mates, duration of adaptation etc.). In this study, the groups were established at weaning and the measurements were carried out once social relationships had stabilized. In this case the individual housing did not result in the physiological signs of stress measured.

The group size had, however, effects on rat behaviour in the open field, singly housed being least active. The result is in agreement with those of *Einon et al.* (1981) and *Angulo et al.* (1991). However, also increased activity in the open field has been reported in individually housed rats (*Gentsche et al.* 1981). Also, individually housed animals and those with one partner in the cage moved slower from the periphery to the central areas. The animals from groups of one and two reared less than the others. The inter-individual variability in the behavioural parameters was largest in rats living alone. The more sluggish behaviour of the singly living rats is perhaps due to their monotonous and eventless environment. The rats living in groups presumably get more stimuli from their cage mates and learn better to cope with the changes in their environment. This conclusion is supported by the finding that individual housing in suspended cages without any handling caused changes in activity, defecation and adrenocortical reactivity in Sprague-Dawley derived rats (*Holson et al.* 1991). Handling for five seconds twice a week eliminated those changes. The lack of social company with other rats is another possible explanation for the behavioural changes observed. According to *Einon et al.* (1978, 1981), isolation of rat pups before 50 days of age has permanent effect upon their later open field behaviour. Rats could be protected from the effects of isolation by short daily periods of social contacts provided that during these periods they are engaged in intense bouts of play. However, only being together with other rats

without play did not inhibit the behavioural changes. This may indicate that the most important impact of cage mates is not to give social company but to give enrichment stimuli.

The cage level in rack changed the latency time of rearing and grooming in the open field. The rats living on the highest shelf of the rack differed most from the others. Presumably light intensity was the most important environmental factor which differed between various rack shelves. The short latency of grooming and long latency of rearing in rats living on the highest shelf may indicate that those animals were habituated to high intensities of light. However, the total frequencies or durations of the grooming or rearing were not affected by the cage level, indicating minor impact of the different environments on the different shelves in this study.

The open field test generates a lot of data. Thus it is hardly surprising that some or several of these measures differ between animals with different histories. However, it seems problematic how to evaluate the importance of these differences; for example, is a factor that affects one open field parameter less important than one that affects two or more? Factor analysis allows the reduction of behavioural variable numbers by combining the correlated variables into one and the same factors. Usually, two general factors are derived from rat behaviour in the open field: 'exploratory activity' or 'motor disharge' and 'emotional reactivity'. The former factor is characterized by ambulation, rearing and activity in the central area, i.e. by behaviour related to lack of fear. 'Emotional reactivity' on the other hand, is characterized by defecation, urination and avoidance of the central area (*Royce* 1977, *Markel et al.* 1989, *Ossenkopp et al.* 1994).

In the factor analysis of this study, Factor 1 can be named 'exploratory' or 'general activity'. It is characterized by activity in the peripheral area, walking and rearing. We did not obtain a clear 'emotional activity' factor; defecation was loaded separately on Factor 6 without any correlation with the other behavioural variables. Activities during the first and last minutes, as well as the rearing, were distributed into several factors which indicates that they had other consequences besides the general activity. Grooming was the only behaviour besides the defecation which was clearly separated from Factor 1. This behaviour is a complex one and can serve functions such as cleaning, counterirritation, thermoregulation, social signalling, increasing and decreasing arousal (*Sachs* 1988). The correlation of grooming with the activity in the last minute indicates that grooming took place mainly during that particular minute. Presumably grooming was related to habituation of the animals to the test situation. The result that the latency of grooming was decreased in rats adapted to high light intensity also supports the idea that grooming in this study illustrated the habituation of the animal.

A factor is a combination of several behavioural measures, thus it should have a stronger discriminative power than any single measure. Moreover, the analysis which takes the animal behaviour comprehensively into consideration, should tell more about the welfare of the animals. In this study, the blocks, group size or cage level did not affect any of the factor scores used, although there were some individual parameters with significant changes. This may indicate that the differences observed were not of great importance for the animal welfare at least on the direct bedding. The effect of gnawing material on the behaviour of rats living without bedding remains to be clarified.

In conclusion, the present study confirms the well known fact that the environment of laboratory animals may have effects on animals and hence on experimental results. Accordingly, it has to be standardized as much as possible, especially in behavioural experiments. The results of the factor analysis, however, indicate that the environ-

mental parameters tested did not have great impact on the welfare of the animals. Moreover, bedding material seems to have some enrichment value for rats, since they did not utilize wooden blocks if they had access to direct bedding. Our results also showed that wooden blocks may decrease the stress of rats adapted to bedding, if they have to be moved to grid floor.

Summary

Han:Wist rats were housed after weaning in groups of one, two, three or four in stainless steel cages with aspen chip bedding, with or without wooden gnawing blocks. The use of the blocks was assessed by video recording and by measuring weight loss of the blocks. Behaviour of the males was tested in a five minute open field test. At the age of 14 weeks the males were transferred into cages with wire mesh bottom without contact bedding. After four weeks, the males were euthanized and weights of the adrenal glands, thymus and spleen were measured. The physiological and behavioural effects of blocks, group size and cage level in rack were tested. In solid bottom cages with direct bedding, the use of the blocks was minimal. It was not affected by the sex or age of the animals. Neither was it affected by the group size or the cage level in a rack. The gnawing of the blocks increased after the rats were transferred on to grid floor without bedding. The food intake or weight gain were not affected by any of the factors studied. The presence of blocks decreased the adrenal weights in rats transferred into wire mesh cages. In open field, the animals living alone were less active and they moved slower from the peripheral to central area than the animals living in groups. The animals living on the highest shelf of the rack differed from the others in their latency times of rearing and grooming. None of the environmental variables tested affected the behavioural factor scores derived from factor analysis. In conclusion, the wooden blocks may reduce the stress of rats adapted to bedding, if they have to be removed to grid floor. The group size or cage level in rack influenced some behaviours of rats in the open field.

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