Effect of housing conditions on behaviour of DBA/2 breeding mice

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Abstract
The impact of housing conditions in different research fields has been studied, but only limited literature has compared the home cage behaviour of breeding pairs under different housing conditions. A previous study showed that the breeding performance was not significantly affected by environmental enrichment (a nest box, a wooden bar for climbing, and nesting material). The aim of the present study was to evaluate the effect of housing conditions on behaviour of paired breeding mice.

Two housing conditions, non-enriched (NE) and enriched (E) housing containing a nest box, a wooden climbing bar and nesting material were studied. A total of 64 DBA/2NCrI BR mice (half of each sex) were used for the experiment. After 4 weeks of adaptation the animals were arranged in breeding pairs and kept in type II L Makrolon cages, 16 pairs for each housing condition. Breeding mice were randomly recorded by video camera from 11 weeks to 36 weeks of age. Behaviours were defined and analysed after data had been collected. Breeding performance and nest quality of each breeding pair were recorded until 40 weeks of age.

In comparison to non-enriched groups enriched animals spent more time on locomotion, digging, eating/drinking, social interaction, while non-enriched mice groomed, mated and jumped more often than enriched groups. Significant housing differences were only found for locomotion behaviour. The frequency of stereotypic behaviour was decreased due to enrichment (1.64% to 0.19%). The nest quality of the E group was significantly better than that of the NE group.

Keywords: DBA/2 mice, breeding pair, environmental enrichment, home cage behaviour

Enrichment has been used as an experimental tool to study the changes in brain functions, immunoreactivity, medical therapy, interactions between environment and genotype, behavioural performances and other parameters. Currently, environmental enrichment is intended for further improvement in laboratory animal housing. Most studies have focused on the effect of housing condition on non-breeding mice (e.g. 1,2,3,4), only a few studies have stressed the influence of enrichment on breeding mice. A previous study5 has shown that the breeding performance was not significantly affected by environmental enrichment (a nest box, a wooden bar for climbing, and nesting material, according to Scharmann6), this being the case for all rack systems (open rack, ventilated cabinet and IVC rack) provided in the study, even though the coefficients of variation in IVC rack (VR-IVC Charles River, Germany) provided were higher for most variables.

The present study focused on the home cage activity/behaviour of breeding mice under two housing conditions in combination with two rack systems (open rack and ventilated cabinet). During the entire experiment half of the breeding pairs had access to a nest box, a wooden climbing bar and nest material, giving them the opportunity to build nests and climb. Home cage behaviours, breeding performance and nest quality were studied.

Material and Methods

Animals and Housing
Animals: In total 32 DBA/2 mice breeding pairs (Charles River Company, the Netherlands) were used for this experiment. After 4 weeks of adaptation at 10 weeks of age, animals were marked and randomly distributed to the two housing systems with 16 breeding pairs per rack system, 8 pairs each for enriched and non-enriched cages. For synchronisation of oestrous cycles (Whitten-effect) some bedding from the male cages was transferred to all female cages one day before animals were regrouped to breeding pairs.
Environment: The animals were kept in two different rack systems: A ventilated cabinet (Scantainer, Scanbur Company, Kage Denmark) and a normal open rack. Both systems were kept in the same animal room under specific pathogen-free (SFF) conditions at a room temperature 22 ± 1°C, with 55 ± 10% relative humidity, a 12/12 hour light/dark cycle and a light intensity of 120-150 Lux (measured 100 cm above the floor).

Housing: All cages were type II elongated Makrolon cages (32.5 x 16.5 x 14 cm, Charles River Company, Sulzfeld Germany). The non-enriched cages (NE group) were provided bedding, food and water. The enriched cages (E group, Figure 1, according to Schramm) contained, in addition to the NE group, a nest box (12 x 7 x 4.5 cm), a wooden bar (13 cm x 7.5 cm, pine) for climbing, and nesting material (nestlets, cotton fibre, 5 x 5 cm, EBECO Company, Castrop-Rauxel, Germany).

Food and water: Tap water in drinking bottles and pelleted food containing 22.5% protein, 5.0% fat, 4.5% fibre and 6.5% ash (Altromin No. 1310, Altromin GmbH, Lage, Germany) were given ad libitum.

Bedding: 70-80 g wood shavings were used for bedding (Altromin Type 3-4, Altromin GmbH, Lage, Germany). Cages and bedding were changed once a week.

Health monitoring: As infections could be the reason for differences in breeding performance and variance, at the end of the experiment the health of the retired breeders was monitored as recommended by FELASA.

Observation of nest quality and behaviour
Before the experiment was performed, the observer was trained by other colleagues. Nest quality and behavioural analysis were scored always by one and the same person. Nest quality was classified according to 5 scales, from one (flat, no visible nest) to five (well performed nest, animals were not seen).

The observing period was separated into three equal phases. Every cage was recorded for 24 hours during each stage. Behaviour was recorded using a time-lapse video recorder (Panasonic AG-TL550E), 24 hours on a 3-hour tape (1 second of the video time equals 8 seconds of the real time). The behavioural data on videotape was viewed, defined and carried out according to the following ethogram (Table 1).

Table 1. Definition of different behaviour

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Resting</td>
<td>Lying or sitting without movement (very short or slight movements are not considered as an interruption).</td>
</tr>
<tr>
<td>Grooming</td>
<td>Shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals.</td>
</tr>
<tr>
<td>Eating</td>
<td>Gnawing food from hopper and bedding.</td>
</tr>
<tr>
<td>Drinking</td>
<td>Standing/sitting beside/under water bottle and licking the drinking nipple.</td>
</tr>
<tr>
<td>Climbing</td>
<td>Jumping onto cage top, climbing along grill in inverted or hanging position.</td>
</tr>
<tr>
<td>Locomotion</td>
<td>Walking, running along, climbing up/down the wooden bar or moving nest material/nest box.</td>
</tr>
<tr>
<td>Digging</td>
<td>Digging into bedding or moving bedding with nose, front paws or hind legs.</td>
</tr>
<tr>
<td>Social contact</td>
<td>Non-aggressive interaction, including moving and playing with each other.</td>
</tr>
<tr>
<td>Mating</td>
<td>Pushing head and forebody beneath a potential mate.</td>
</tr>
<tr>
<td>Jumping</td>
<td>Jumping towards the cage top vertically along the wall.</td>
</tr>
<tr>
<td>Stereotype</td>
<td>Consecutively and repeatedly climbing, digging and jumping in the same position (for more than 30 seconds).</td>
</tr>
<tr>
<td>Others</td>
<td>Except the behaviours described above.</td>
</tr>
</tbody>
</table>
**Figure 2.** The time budget (%) of different behaviours (left: NE group, right: E group)

**Experimental design**
Following 4 weeks of adaptation at 10 weeks of age the animals were marked and randomly separated to the two rack systems described above. Breeding pairs were kept together (one pair/cage) during the entire experimental period from November to February.

After mice had been regrouped to one breeding pair per cage, breeding pairs were randomly recorded by video camera (Panasonic, AG-TL50E) until 36 weeks of age (each cage was recorded for 3x24 hours). Nest quality was graded every second day between 10:00 and 12:00. Breeding performance was recorded until 40 weeks of age, including litter size, number of pups weaned and body weight at weaning. Behaviours were defined and analysed following data collection. Time budgets were then constructed using the definitions given in Table 1. A mean value per cage was calculated.

**Statistics**
Data were analysed by StatView 5.0 software (SAS Institute Inc., Cary NC, USA, 1998). The normal distributed data were compared using a two-factorial analysis of variance with the factors 'rack system' and 'housing' to analyse the effects of the rack systems, the housing and the rack systems x housing interaction, with a significance level of 5%. Non-parametric tests, Mann-Whitney test, were performed for abnormally distributed data.

Two females, one from each housing type, died during the experiment. Therefore, the data were not included in the statistical analysis. The average duration of all behaviours of the two mice in the same cage was used for statistical purposes (n is equal to the cage number). The data of stereotypic behaviour were separated for three phases for detailed information.

**Results**

**The effects of housing condition on home cage behaviours**
In comparison to animals in the NE groups enriched animals spent more time on locomotion, climbing.

**Figure 3.** Relative duration (%) of home cage behaviours (n=15, ***: p< 0.001)
digging, social interaction, while non-enriched mice groomed, jumped and mated more often than the enriched group (Figure 2). Significant housing differences between the NE and E groups were found only in locomotive behaviour \( (F_{1,36} = 18.152, p = 0.0002, \text{ Figure 3}). \)

**The effects of rack system on behaviours**

Animals kept in both rack systems showed similar relative duration (%), except resting, climbing and locomotion behaviour (Figure 4). Animals kept in open racks slept more (63.54±3.38%) than animals in ventilated cabinets (58.33±3.21%), this being observed for both housing conditions \( (F_{1,36} = 8.659, p = 0.0068). \) Mice kept in ventilated cabinets climbed more and showed more locomotion than those in open racks \( (F_{1,36} = 9.054, p = 0.0058 \text{ for climbing}; \ F_{1,36} = 6.316, p = 0.0185 \text{ for locomotion}). \) No rack system and housing interaction was found.

**The effects of housing condition on stereotypic behaviour**

Stereotypic behaviours, including stereotypic jumping, climbing and digging, were found more often in the non-enriched groups (5 cages in NE and 2 cages in E group) at the beginning of the experiment, the frequency of stereotyping observed decreasing with time (Figure 5). In the 3rd experimental period only one cage from each group showed any stereotypic behaviour. On average the duration of stereotypic behaviour was 1.64% in NE groups and 0.19% in E groups during the entire experiment, but no statistical difference between the groups could be detected.

**Nest quality and breeding performance**

Nest quality of E groups was better than that of NE groups (median: scale 2.000 for NE groups and scale 4.000 for E groups), this leading to a significant housing difference (Mann-Whitney test, \( p < 0.0001 \)).

**Figure 4.** The relative duration (%) of different behaviours in different rack systems

(OR: open rack, S: ventilated cabinet; ***: \( p < 0.001, **: p < 0.05; n = 15 \))

**Figure 5.** Relative duration (%) of stereotypic behaviour during three different phases \( (n = 15) \)
The breeding performance of both housing groups was similar; no housing effect was found (data are not shown).

Discussion

Early studies have shown that providing nest material can lead to a better breeding performance in rats. This was not supported in mice by previous studies and the present study. Tsai et al. reported that enriched housing caused a delay in the age of dam at first weaning in all rack systems (open rack, ventilated cabinet and IVC rack). The present study showed that enriched animals spent less time on mating behaviour and more time on locomotion/digging/climbing/social contact (Figure 2) in comparison to the NE group. The results indicate that playing and investigating the environment were more attractive for the animals kept in E cages. This may be the reason why enriched housing did not improve the breeding index.

An interesting finding is the difference between rack systems. The animals in open racks slept significantly longer than those in ventilated cabinets and showed significantly less climbing and locomotion behaviour. For this finding one possible explanation is that mice in open racks were not disturbed by vibrations compared with mice in ventilated cabinets. We could not find other reasonable explanations. To understand this phenomenon further studies are necessary.

The stereotypes may serve as general “coping mechanisms” (e.g., reviewed in Mason & Latham). The present study found that the frequency of stereotypic behaviours decreased with time. Thus, it can be assumed that breeding mice need some time to adapt to their environment and enriched housing provided for the experiment may help to decrease this time needed for adaptation. Nevison et al. reported that enriched housing (nest material and tunnel) can increase and decrease stereotypes in non-breeding mice; the results however varied according to the strain. The enriched housing significantly increased the stereotypic behaviour of DBA/2 mice. This indicates that nest material and a tunnel are not suitable for non-breeding DBA/2 mice.

According to the presented data, it can be assumed that enriched housing used in this study may reduce the stereotypic behaviour of breeding DBA/2 mice, at least in helping them cope with environmental or regrouping stress. Furthermore, the effects of enrichment could be different, these varying due to the enrichment given and the animals used in a study.

References

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