Are the effects of different enrichment designs on the physiology and behaviour of DBA/2 mice consistent?

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Summary

Environmental enrichment is intended to improve the well-being of laboratory animals. Although many researchers have indicated that environmental enrichment may enhance animal well-being, there is some evidence that enrichment differs in its effects on physiology and behaviour between species and strains. The present study focuses on the effects of different enrichment designs on the physiology and behaviour of male and female DBA/2 mice. A total of 48 DBA/2J mice, 24 males and 24 females were used for this experiment. Upon arrival at about 3 weeks of age, the animals were randomly allotted to three experimental groups: NE, non-enrichment; E1, enriched with nest box, wooden climbing bar and nest material according to Scharmann (1993); E2, enriched with horizontal and vertical dividers, modified from Haemisch and Gärtner (1994). Same-sex groups of four mice were housed for 12 weeks in type III Makrolon cages with (E1 or E2) or without (NE) enrichment objects. Behavioural performance (Open Field, Food Drive and Elevated Plus Maze tests) and physiological traits (haematological variables, body weight and organ weights, corticosterone and thyroxine levels) were measured. This study observed that enrichment had significant effects on the mean values of body weight (females), Open Field and Food Drive tests. The most significant housing differences were found between the E2 and NE/E1 groups. Furthermore, sex differences in the NE, E1 and E2 groups were not consistent for several variables (growth rate, relative weights of spleen, kidney and heart, Food Drive and Elevated Plus Maze behavioural performance). There was often a higher coefficient of variation (CV) in the E1 and E2 groups as compared to the NE group, chiefly in physiological traits and in the Open Field and Food Drive tests. The results of this study indicate, that the effects of enrichment designs used in the present study are not consistent, but vary according to sex and the variable studied.

Keywords  DBA/2 mice; environmental enrichment; behavioural test; haematology; organ weights

The correlations or interactions of environment and genotype are regarded as part of the environmental variance responsible for the variability of quantitative characteristics of laboratory animals (Gärtner et al. 1979).

Enrichment is currently considered a popular means for improving animal well-being, and various enrichment designs have been recommended on the basis of preference tests. The evaluation of enrichment has mostly been focused on the effects on the mean values of behavioural performances, brain functions, immunoreactivity, organ weights and other parameters (Manosevitz &

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Accepted 29 April 2003

A few studies have stressed the effect of enrichment on the variation instead of mean values. Purves (1996) reported that enrichment reduced the variability of research results, while other scientists (Eskola et al. 1999, Gärtner 1999, Nevalainen et al. 1999, Tsai & Hackbarth 1999, Mering 2001) have indicated that enrichment might enhance variation, and thereby result in higher numbers of animals being needed for some experiments (e.g. organ weights, aluminium-foil behavioural performance and clinical chemistry parameters such as triglycerides, corticosterone).

In previous studies, Tsai et al. (2002, 2003) observed that body weight, haematological data, organ weights and breeding index were not significantly influenced by the enrichment design (nest box, wooden climbing bar and nest material according to Scharmann 1993), but that enrichment apparently did have an effect on the variation. These effects are strain- and test-dependent. Furthermore the strain differences in body weight and haematological data were also affected by Scharmann’s design.

Although a wide variety of enrichment items and materials is available, there is still only limited information about the effects of different enrichment designs on mean values and variation. In addition DBA/2 mice is a widely used strain, but there is a lack of information about the effects of environmental enrichment on experimental results of DBA/2 mice. Thus the present study focused on the effects of different enrichment designs on physiological traits and behavioural performances of DBA/2 male and female mice.

Material and methods

Animals

In total, 48 DBA/2 mice (24 males and 24 females) aged about 3 weeks obtained from Elevage Janvier (Le Genest, St Isle, France), were marked by ear puncturing and randomly allotted to three experimental groups, with equal numbers of cages in same-sex groups of four.

Environment

All animals were maintained in the same animal room at a room temperature of 21 ± 1°C and 50 ± 10% relative humidity, on a 12/12 h light/dark cycle (light on at 06:00 h) and at a light intensity of 150 ± 10 Lux (measured 100 cm above floor level).

Housing

Two enrichment designs were used in this experiment, as shown in Fig 1. Enrichment design 1 (E1 group according to Scharmann 1993) contained a nest box (16 cm × 22 cm × 4.5 cm) constructed out of the bottom of a type II Makrolon cage as well as a wooden climbing bar (16.5 × 8.5 cm, pine) and nesting material [cotton fibre Nestlets, 5 cm × 5 cm, EBECO, Castrop-Rauxel, Germany]. Enrichment design 2 (E2 group) contained horizontal and vertical clear safety glass dividers (5 cm high), modified from Haemisch and Gärtner (1994). All animals were kept in type III Makrolon cages (Scanbur, Køge, Denmark) (37.5 × 21.5 × 15 cm), with (E1 or E2) or without (NE) enrichment.

Food and water

Tap water in drinking bottles and pelleted food containing 19.0% protein, 4.0% fat, 6% fibre and 7% ash (Altromin No. 1324, Altromin GmbH, Lage, Germany) were given ad libitum.

Bedding

As bedding, 130–140 g soft wood shavings were provided for each cage (Altromin type 3–4, Altromin GmbH, Lage, Germany). Cage and bedding were changed once a week (always on Thursdays).

Test methods

Test and sampling order

All tests and samplings were done sequentially in groups of three using one animal
from each housing group (NE, E1 and E2) until all of the animals had been tested or sampled. Test or sampling of the same variables was completed within one week.

**Behavioural tests**

Three behavioural tests, Open Field, Food Drive (modified Open Field test) and Elevated Plus Maze were chosen for this study, as they were developed for testing the drug effect of anxiolytics in pharmacological research.

**Open Field:** The Open Field method used here was modified from Thompson [1953] and Price and Stokes [1975]. A 60 x 60 cm board was divided into 64 numbered squares and enclosed by a wall 50 cm high. At the beginning of the test, each animal was placed in the central area as shown in Fig 2. The animal’s position was recorded every 4 s for 5 min by the number of the squares occupied. Mice were tested between 09:00 and 12:00 h. The board was cleaned with fresh water after each animal was tested. The light intensity on the board was 2 ± 1 Lux.

**Definition:**

- Central area and corner are given in Fig 2.
- The position of the animal was recorded as the square where the animal had placed its right hind leg.
- Travel distance (crossing, cm) was measured between the centres of two recorded squares within 4 s.
- Freezing is defined as a period when the animal stays in the same square for more than 4 s.

**Food Drive:** The Food Drive behavioural test used here was modified from Rex et al. [1996] using the same device as for the Open Field test. Ten pellets of fresh food were placed in the central area as shown in Fig 2. The first touching time and the eating time were recorded during the test.

Before starting the test, food was withheld for 12 h, but water was available. During this time a piece of paper towel was substituted for normal bedding. The same test order as described above was always observed.

The animals were placed in the same corner at the start. The test was stopped.
when the animals had not touched the food within 15 min. The board was cleaned between each test. The animals were tested between 09:00 and 13:00 h. The light intensity on the board was 2±1 Lux.

**Definition:**
- First touching time (seconds) is the time elapsed until the animal came to the central area and touched the food pellets for the first time.
- Eating time (seconds) was recorded as the time the animal stayed in the central area holding the pellet continuously for more than 30 s.

**Elevated Plus Maze:** The Elevated Plus Maze method used here was modified from Dawson and Tricklebank (1995) and Montkowski et al. (1997). The Elevated Plus Maze was made of plastics coated board (opaque) and placed on a stand 80 cm high. The test board consisted of four 5×30 cm long arms, two open and two enclosed, arranged in the form of a plus (Fig 3). The closed arms were surrounded on three sides by walls 25 cm high. The central area measured 5×5 cm. The light intensity on the board was 4±1 Lux in the open arms, 2 Lux in the central area and 1±1 Lux in the closed arms.

Animals were placed in the central area facing one open arm. The total time in seconds spent by the animals in either arm was recorded over a period of 5 min, as was the frequency of entering open and closed arms and the central area. Following the experiment the average time (seconds) spent in each area per entry was calculated.

**Definition:**
- The area where the animal stayed was recorded when the mouse had placed both front legs inside the area.

**Haematological analysis**
Twenty microlitres of blood was sampled by retrobulbar venous puncture under light ether anaesthesia with 20 μl, heparin-coated capillary (Hirschmann Laborgeräte, Germany) and slowly mixed with dilute buffer (Heama-Line DIFF, Biochem Immunosystems Inc., Allentown, PA, USA); the total volume was 20 ml. Diluted blood samples were analysed by the Cell Counter System 9000 (Serono-Baker Diagnostics, Allentown, PA, USA). The items analysed included white blood cell count \(\text{WBC}, \times 10^9/\text{l}\), the red blood cell count (RBC,
10^12/l], the concentration of haemoglobin (HGB, mmol/l) and the haematocrit (HCT, %).

**Euthanasia**

At 15 weeks of age mice were moved from the animal room to the laboratory (within 30 s) and euthanized in their home cages. The cage cover was replaced by a safety glass lid with a hole for a tube through which CO₂ was introduced into the cage at a flow rate of 6 l/min (Hackbarth et al. 2000). All animals lost consciousness within 30 s and stopped breathing within 2–3 min after CO₂ was introduced into the cages.

To minimize the effect of sampling order on the values of corticosterone and thyroxine (described by Gärtner et al. 1980, Shanks et al. 1990, Dahlborn et al. 1996), four mice were euthanized together in their home cage and sampling was done alternating in groups of one cage per each group (NE, E1, E2). The sampling order was unalterable until all of the animals had been euthanized.

**Corticosterone (CORT) and thyroxine (T4)**

Blood samples were collected by heart puncture directly after euthanasia (7 days after haematological analysis). After centrifuging the serum was pipetted into Eppendorf vials (0.5 ml, Landgraf Laborgeräte, Langenhagen, Germany) and frozen at −20°C. The corticosterone (ng/ml) and thyroxine (nmol/l) concentrations were measured using a commercially available radioimmunoassay (No. TKRC1 and No. TKT41, Diagnostic Products Corporation, Los Angeles, CA, USA) in the Institute of Analytical Chemistry and Endocrinology of the School of Veterinary Medicine, Hannover.

**Termination procedures**

Following euthanasia, body weight was recorded, followed by blood sampling and weighing of organs (heart, liver, kidney, adrenal, spleen and uterus). After dissection the organs were kept in a wet chamber (Petri dish with wet cotton) until weighing. Body weights were determined using an electronic balance (Item number: EOD110, Ohaus Corporation, Florham Park, USA), but a more sensitive balance (ST-200, Denver Instrument Company, Denver, CO, USA) was used for organ weights.

**Health monitoring**

Sentinel animals were kept in the same animal room using open cages and provided with dirty bedding from other cages during the entire experimental period. They were checked for and declared free from all bacteria and parasites listed in the FELASA recommendation (Nicklas et al. 2002) and the following viruses: MHV, rotavirus, paroviruses (MVM, MPV), PVM, Sendai and Theiler’s murine encephalomyelitis.

**Experimental design**

Following one week of adaptation body weight, food intake and water intake were recorded weekly from 4 weeks until 15 weeks of age. Open Field, Food Drive and Elevated Plus Maze behavioural tests were performed at 9, 10 and 11 weeks of age, respectively. Blood samples were collected at 14 weeks of age and WBC, RBC, HGB and HCT were determined.

At 15 weeks of age body weights were recorded (before blood sampling) and blood samples, for CORT and T4 measurement, were collected. Organ weights (heart, liver, kidney, adrenal, spleen and uterus) were measured after blood sampling. Health monitoring was performed at the end of the experiment using sentinel animals.

**Statistics**

Data were analysed using StatView software (version 5.0, SAS Institute Inc., Cary, NC, USA, 1998). The percentage of body weight gain during the entire experiment was calculated as follows for comparing the growth rate (%) in the different housing conditions: [(final body weight – body weight at 3 weeks of age)/body weight at 3 weeks of age].

Body weights during the entire experiment were compared using repeat-measures ANOVA. The mean values of each cage of behavioural performances and growth rate and the total food and water intakes of each
cage were calculated and compared using a two-factorial analysis of variance with the factors ‘sex’ and ‘housing’, followed by the Scheffé test [significance level 5%] to analyse the effect of housing and the response of sex under different housing conditions (Lee 1999).

To achieve independence from mean values, the coefficients of variation [SD/mean value] within cages were used instead of the variance [SD^2] to compare the variation between the NE and E1 or between the NE and E2 groups. The CV of each cage was calculated separately. The average CVs of each variable were pooled and compared using the Wilcoxon signed rank test (non-parametric pair t-test). As the CVs were not distributed normally, the median was calculated.

**Results**

*The effect of enrichment on body weight and growth rate*

The body weights in different housing groups were computed for each week as shown in Figs 4 and 5.

There was no significant housing difference in body weight in male groups during the entire experiment, while a significant housing difference was found in females [F_{2,180} = 3.539, P = 0.0454], mainly due to the difference between the NE and E2 mice (Scheffé test P = 0.0455). A significant sex difference [F_{1,360} = 106.437, P ≤ 0.0001] was observed in body weight, but no significant housing–sex interaction was found.

Both enriched male groups had higher growth rates (percentage of body weight gain) than the NE group, while both enriched female groups showed lower growth rates in comparison to the NE group [Fig 6]. There was a significant housing–sex interaction in growth rate [F_{2,6} = 8.876, P = 0.0161], as a significant sex difference was only found in the E2 group (Scheffé test P = 0.0054), but there was no sex difference in the NE and E1 groups.

*Food and water intake*

A higher food intake was observed in the NE group [109.6 g/week], than in the E1 (99.7 g/week) and E2 (95.2 g/week) groups. There was a significant housing difference [F_{2,6} = 13.196, P = 0.0064], but no significant sex difference or housing–sex interaction was found.

Similar to food intake, a higher water intake was observed in the NE group [125.2 ml/week in the NE group, 117.9 ml/week in the E1 group and 110.6 ml/week in the E2 group] and this led to a significant housing difference in water intake [F_{2,6} = 13.246, P = 0.0063]. No significant sex difference or housing–sex interaction was observed.

![Fig 4](image-url) **Body weight of DBA/2 males versus age.** NE = non-enriched group; E1 = enriched group according to Scharmann (1993); E2 = enriched group, modified from Haemisch and Gärtner (1994)
The effect of enrichment on behavioural performance

Significant housing differences were found in the Open Field and Food Drive performances (Tables 1 and 2). There was no significant housing difference in the Elevated Plus Maze test (data not shown).

In the Open Field test, both enriched male groups showed higher activity (travel distance) and less freezing in comparison to the NE group. Female E1 mice showed a lower activity and higher freezing frequency than the NE mice, while the E2 mice had increased travel distance and reduced freezing frequency in comparison to the NE mice (Table 1).

There is a significant housing difference in travel distance \((F_{2,6} = 14.905, P = 0.0047)\) due to the differences between the NE and E2 mice (Scheffé test \(P = 0.0135)\) and the E1 and E2 mice (Scheffé test \(P = 0.0072)\). A significant housing difference was also found in freezing frequency \((F_{2,6} = 9.942, P = 0.0125)\) due to the difference between the NE and E2 mice (Scheffé test \(P = 0.0236)\) and that between the E1 and E2 mice (Scheffé test \(P = 0.0236)\). No significant sex difference or housing–sex interaction was found in Open Field performance.
During the Food Drive test, males of the E1 group needed more time to reach the central area to touch food, while mice of the E2 group needed less time than those of the NE group. Both enriched groups started to eat food later than the NE group. The difference between the NE and E1 groups was larger than that between the NE and E2 groups (Table 2).

Female mice of the E1 and E2 groups required more time before they reached the central area to touch or to eat food. Mice of the E2 group touched food earlier than mice of the E1 group, but started to eat food later than those of the E1 group (Table 2).

There was a significant housing difference in the first touching time \( F_{2,6} = 6.605, P = 0.0047 \), mainly due to the difference between the E1 and E2 groups (Scheffé test \( P = 0.0334 \)). A significant sex difference was also observed in the first touching time \( F_{1,6} = 32.208, P = 0.0013 \), due to the sex difference of the E1 group (Scheffé test \( P = 0.0231 \)) (Fig 7). No housing–sex interaction was found in Food Drive performance.

Inconsistent sex differences in the NE, E1 and E2 groups were also observed in the Elevated Plus Maze test (Figs 8 and 9). Significant sex differences were found in the E2 groups (Scheffé test \( P = 0.0268 \) for the time spent in a closed arm per entry; Scheffé test \( P = 0.0057 \) for the time spent in the central area per entry, Figs 8 and 9), while no significant sex differences were found in the NE and E1 groups. A significant housing–sex interaction was found for the time spent in a closed arm per entry \( F_{2,6} = 4.424, P = 0.0225 \).

### Table 1 The mean value \( \pm SD \) of Open Field behavioural performance

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Housing</th>
<th>Female</th>
<th>Male</th>
<th>Housing difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Travel distance (cm)</td>
<td>NE</td>
<td>1646.3 ( \pm ) 241.4</td>
<td>1470.8 ( \pm ) 246.2</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td></td>
<td>E1</td>
<td>1426.5 ( \pm ) 337.2</td>
<td>1564.4 ( \pm ) 405.5</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>2017.8 ( \pm ) 391.6</td>
<td>1971.6 ( \pm ) 268.5</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td>Freezing (frequency)</td>
<td>NE</td>
<td>21.8 ( \pm ) 2.4</td>
<td>24.6 ( \pm ) 3.7</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td></td>
<td>E1</td>
<td>23.5 ( \pm ) 9.1</td>
<td>22.9 ( \pm ) 8.0</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>15.8 ( \pm ) 4.9</td>
<td>18.0 ( \pm ) 6.9</td>
<td>( P = 0.0047 ) s</td>
</tr>
</tbody>
</table>

NE: non-enriched group; E1: enriched group according to Scharmann (1993); E2: enriched group, modified from Haemisch and Gärtnert (1994)

s: significant difference

### Table 2 The mean value \( \pm SD \) of Food Drive behavioural performance

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Housing</th>
<th>Female</th>
<th>Male</th>
<th>Housing difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>First touching (s)</td>
<td>NE</td>
<td>43.0 ( \pm ) 14.8</td>
<td>83.6 ( \pm ) 19.1</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td></td>
<td>E1</td>
<td>53.8 ( \pm ) 17.9</td>
<td>113.9 ( \pm ) 81.2</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>45.9 ( \pm ) 23.5</td>
<td>62.0 ( \pm ) 21.5</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td>Eating (s)</td>
<td>NE</td>
<td>211.1 ( \pm ) 97.0</td>
<td>209.9 ( \pm ) 51.0</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td></td>
<td>E1</td>
<td>257.9 ( \pm ) 220.1</td>
<td>447.8 ( \pm ) 349.0</td>
<td>( P = 0.0047 ) s</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>321.1 ( \pm ) 180.8</td>
<td>265.9 ( \pm ) 168.0</td>
<td>( P = 0.0047 ) s</td>
</tr>
</tbody>
</table>

NE: non-enriched group; E1: enriched group according to Scharmann (1993); E2: enriched group, modified from Haemisch and Gärtnert (1994)

s: significant difference

The effects of enrichment on haematological values and relative organ weights

There was no significant effect in haematological values or in relative organ weights due to housing (data not shown).

Inconsistent sex differences under the three housing groups were noted in relative weights of liver, kidney and spleen (see Table 3), but not in the haematological values (data

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Fig 7  Interaction graph for Food Drive performance (first touching time). NE = non-enriched group; E1 = enriched group according to Scharmann (1993); E2 = enriched group, modified from Haemisch and Gärtnert (1994); s = significant difference; n.s. = no significant difference

Fig 8  Interaction graph for Elevated Plus Maze performance (in a closed arm per entry). NE = non-enriched group; E1 = enriched group according to Scharmann (1993); E2 = enriched group, modified from Haemisch and Gärtnert (1994); s = significant difference; n.s. = no significant difference

Fig 9  Interaction graph for Elevated Plus Maze performance (in the central area per entry). NE = non-enriched group; E1 = enriched group according to Scharmann (1993); E2 = enriched group, modified from Haemisch and Gärtnert (1994); s = significant difference; n.s. = no significant difference
not shown). A significant housing-sex interaction was found for the relative heart weights ($F_{2,6} = 6.579$, $P = 0.0307$).

**T4 and CORT levels under different housing conditions**

Male mice had significantly higher T4 levels than female mice ($F_{1,6} = 9.937$, $P = 0.0198$) [Fig 10]. No significant housing difference was found for these parameters.

For CORT concentration [Fig 11] there was a significant difference between the sexes ($F_{1,6} = 5.048$, $P = 0.0300$), but no significant housing difference. It should, however, be mentioned that the difference between male NE and E1 mice was close to a significant level (Scheffé test $P = 0.0581$).

**The effect of enrichment on the CV**

There was a higher tendency towards an increased CV in both enriched groups, as opposed to the NE group (Tables 4 and 5). The median CVs of the NE, E1 and E2 groups were 12.9%, 15.8% and 16.6%, respectively; the interquartile ranges of the NE, E1 and E2 groups were 16.6, 22.6 and 16.0. The CVs of each housing condition were pooled and compared using the Wilcoxon signed rank test (non-parametric pair t-test). Significant differences were detected between the NE and E1 groups ($P = 0.0170$), chiefly in the Open Field and Food Drive tests ($P = 0.0173$) and between the NE and E2 groups ($P = 0.0062$), mainly for the haematological parameters ($P = 0.0117$) and for the Open Field and Food Drive performances ($P = 0.0173$).

**Discussion**

The present study demonstrated that enrichment affected the results of the Open Field and Food Drive tests. Significant housing differences were observed between the NE and E2 groups and between the E1 and E2 groups, while no significant housing
### Table 4  The coefficient of variation (%) of behaviour performance

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sex</th>
<th>NE</th>
<th>E1</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Travel distance</td>
<td>Female</td>
<td>14.5</td>
<td>23.6</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>17.1</td>
<td>25.4</td>
<td>13.7</td>
</tr>
<tr>
<td>Freezing times</td>
<td>Female</td>
<td>11.5</td>
<td>38.9</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>14.6</td>
<td>35.1</td>
<td>38.7</td>
</tr>
<tr>
<td>First touching</td>
<td>Female</td>
<td>34.0</td>
<td>31.5</td>
<td>50.9</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>24.9</td>
<td>73.8</td>
<td>35.4</td>
</tr>
<tr>
<td>Eating</td>
<td>Female</td>
<td>45.7</td>
<td>83.3</td>
<td>57.0</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>25.5</td>
<td>79.9</td>
<td>67.5</td>
</tr>
<tr>
<td>In closed arm</td>
<td>Female</td>
<td>8.8</td>
<td>11.3</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>11.8</td>
<td>15.1</td>
<td>8.6</td>
</tr>
<tr>
<td>In central area</td>
<td>Female</td>
<td>29.5</td>
<td>24.4</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>22.6</td>
<td>22.9</td>
<td>24.5</td>
</tr>
<tr>
<td>In open arm</td>
<td>Female</td>
<td>53.8</td>
<td>63.1</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>69.6</td>
<td>66.3</td>
<td>71.7</td>
</tr>
<tr>
<td>In closed arm/entry</td>
<td>Female</td>
<td>19.5</td>
<td>19.6</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>13.0</td>
<td>40.8</td>
<td>21.9</td>
</tr>
<tr>
<td>In central/entry</td>
<td>Female</td>
<td>26.0</td>
<td>22.9</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>22.8</td>
<td>16.7</td>
<td>22.0</td>
</tr>
<tr>
<td>In open arm/entry</td>
<td>Female</td>
<td>40.6</td>
<td>44.3</td>
<td>66.9</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>23.3</td>
<td>28.8</td>
<td>28.3</td>
</tr>
</tbody>
</table>

NE: non-enriched group; E1: enriched group according to Scharmann (1993); E2: enriched group, modified from Haemisch and Gärtner (1994). In closed arm, in central area or in open arm: the total time in seconds spent by the animals in either area during Elevated Plus Maze test. In closed arm/entry, in central area/entry or in open arm/entry: the average time in seconds spent by the animals in either area per entry during Elevated Plus Maze test.

### Table 5  The coefficient of variation (%) of physiological traits

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sex</th>
<th>NE</th>
<th>E1</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>Female</td>
<td>7.1</td>
<td>14.1</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>13.2</td>
<td>25.6</td>
<td>23.7</td>
</tr>
<tr>
<td>RBC</td>
<td>Female</td>
<td>2.3</td>
<td>3.1</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>6.3</td>
<td>5.8</td>
<td>11.0</td>
</tr>
<tr>
<td>HGB</td>
<td>Female</td>
<td>2.5</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>5.2</td>
<td>5.3</td>
<td>10.1</td>
</tr>
<tr>
<td>HCT</td>
<td>Female</td>
<td>2.6</td>
<td>2.3</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>6.0</td>
<td>5.6</td>
<td>11.3</td>
</tr>
<tr>
<td>Final body weight</td>
<td>Female</td>
<td>12.9</td>
<td>13.8</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>7.3</td>
<td>8.7</td>
<td>9.3</td>
</tr>
<tr>
<td>Relative liver weight</td>
<td>Female</td>
<td>4.3</td>
<td>5.5</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.1</td>
<td>5.6</td>
<td>5.9</td>
</tr>
<tr>
<td>Relative kidney weight</td>
<td>Female</td>
<td>5.0</td>
<td>3.5</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>9.6</td>
<td>4.5</td>
<td>9.4</td>
</tr>
<tr>
<td>Relative spleen weight</td>
<td>Female</td>
<td>6.7</td>
<td>8.1</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>12.5</td>
<td>14.6</td>
<td>20.2</td>
</tr>
<tr>
<td>Relative adrenal weight</td>
<td>Female</td>
<td>28.4</td>
<td>12.2</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>22.2</td>
<td>16.6</td>
<td>19.8</td>
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<td>Relative heart weight</td>
<td>Female</td>
<td>7.9</td>
<td>9.6</td>
<td>7.0</td>
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<tr>
<td></td>
<td>Male</td>
<td>9.3</td>
<td>6.2</td>
<td>8.7</td>
</tr>
<tr>
<td>Relative uterus weight</td>
<td>Female</td>
<td>19.9</td>
<td>24.0</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>5.0</td>
<td>8.7</td>
<td>10.2</td>
</tr>
</tbody>
</table>

NE: non-enriched group; E1: enriched group according to Scharmann (1993); E2: enriched group, modified from Haemisch and Gärtner (1994). WBC = white blood cells, RBC = red blood cells, HGB = haemoglobin, HCT = haematocrit.
difference was found between the NE and E1 groups. This indicates that the effects of the two enrichment designs (E1 and E2) on the results of the Open Field and Food Drive tests are not consistent. This is comparable to the results of the Open Field and Food Drive tests on the two enrichment designs (E1 and E2) on the groups. This indicates that the effects of the difference was found between the NE and E1 addition, Nevison et al. 1996, Van de Weerd et al. 1997, Eskola & Kaliste-Korhonen 1999, Tsai et al. 2002). A significant housing difference was found only in body weight in females.

The present study observed different reaction patterns to the housing conditions in the growth rate of male and female mice, though a significantly higher food intake was found in both NE groups. Van de Weerd et al. 1997 assumed that the heat loss of enriched mice was reduced because of nesting material, accounting for the fact that animals consumed less food. According to the present results heat loss reduction may not be the only reason. Other factors, such as non-enriched mice spending more time playing with food, and the different effects of enrichment on the social structures of males and females, may be also involved.

The present study shows that E1 led to increased differences between the sexes in Food Drive performance, relative spleen weight and relative heart weight, while E2 resulted in enhanced differences between the sexes in the growth rate, Elevated Plus Maze performance and in relative spleen weight, and in a reduced difference between the sexes in relative kidney weight. This indicates that the sexes reacted differently to the different enrichment designs (E1 and E2), though significant housing–sex interactions were only found in the growth rate, the Elevated Plus Maze and in the relative heart weight.

Similarly, Haemisch and Gärtnert 1994 reported that enriched DBA/2 male mice had higher serum corticosterone levels. In addition, Nevison et al. 1999 found that DBA/2 males provided with a clear Perspex tunnel and shredded tissue showed increased stereotypic behaviour as well as higher serum corticosterone levels. Peng et al. 1989 reported that social hierarchy development might play an important role in animal stress and that fighting between mice within newly assigned groups may be the major cause of the elevated corticosteroid concentrations. Haemisch and Gärtnert 1994 observed that changes of dominant positions occurred more often in enriched male groups, leading to intensified aggression in the enriched cages. Such altered social organization was found to correspond with corticosterone titres. The corticosterone levels of this study showed that enrichment (E1 and E2) resulted in effects contradictory between two sexes. Factors such as different social hierarchy development and the changes in dominant positions may be involved. To explain the different effects on the two sexes, further study is needed.

The present study shows that environmental enrichment has different influences on CV, depending on the sex and the variables studied. In general, both enriched groups showed higher CV for most physiological traits and for the Open Field and Food Drive tests. Such effects have already been published or can be recalculated from the data of previous reports (Thiessen et al. 1962, Manosevitz & Pryor 1975, Hull et al. 1976, Haemisch & Gärtnert 1994, Bergmann et al. 1994/95, Dahlborn et al. 1996, Van de Weerd et al. 1997, Eskola & Kaliste-Korhonen 1999, Nevison et al. 1999, Roy et al. 2001, Tsai et al. 2002).

Currently, providing different housing conditions for a given experiment is considered to give more generalizable and valid results, because standardization of conditions may decrease the external validity of data owing to results being highly idiosyncratic. According to our results, the number of animals may need to be increased in such studies using DBA/2 mice with enriched housing conditions (such as ours) in which the focus is on physiological traits and Open Field and Food Drive tests.

In conclusion the present study demonstrated significant housing effects on body weight (females) as well as on the Open Field and Food Drive performances, but there were no significant effects of either enrichment
designs used on the other parameters studied: physiological traits (haematological data and organ weights) and Elevated Plus Maze test. Housing differences were found for body weight (females) and behavioural performance (Open Field and Food Drive tests), mainly due to the differences between the E2 and NE/E1 groups. Sex differences of the NE, E1 and E2 groups were not consistent for growth rate, Food Drive, Elevated Plus Maze performance or relative weights of spleen, kidney and heart. This shows that the effects of enrichment designs (E1 and E2) were not consistent on DBA/2 mice.

Both enrichments led to enhanced variation in most variables, especially physiological traits and the Open Field and Food Drive tests.

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