Importance and Effects of Enrichment on Physiology, Behaviour and Breeding Performance in Mice

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Summary
Sixty DBA/2 breeding pairs were used to understand the influence of enrichment on breeding performance. Four inbred strains of mice (AJ, BALB/c, C57BL/6 and DBA/2) were used for sequential experiments, in a total number of 240 (half for each sex), to collect information about the effect of enrichment on physiological traits and behavioural tests. Non-enriched and enriched groups were always provided with the same cage type to avoid the influence of floor space on agonistic behaviour, which can lead to different inter-individual distances.

According to the presented results environmental enrichment can affect experimental results and will not automatically improve well-being. Furthermore “Reduction” and “Refinement” may conflict with each other. Thus it will be necessary to evaluate the effects of environmental enrichment before an enrichment design is applied for a given experiment.

Keywords: mice, enrichment, variation, refinement and reduction.

Introduction
In recent decades, there have been significant developments in laboratory animal science in the field of health monitoring (hygiene control), genetics and environment. This led to the possibility of standardisation in laboratory animal science. Reduction and refinement have been implemented in laboratory animal science in the last decades. Due to that the high level of standardisation has enhanced animal well-being by improving physiological health (refinement) and is largely responsible for the great decrease in the number of laboratory animals used in experimental research, due to the decrease in variation (reduction).

Currently environmental enrichment is intended for further improvement on the laboratory animal housing. Due to that a wide variety of enrichment designs are considered and the interactions between environment and genotype have been reported in many studies. The evaluation of enrichment has mostly been focused on the effects of experimental results (mean values) on the brain functions, behavioural performances and other parameters.

Concerning the reduction of 3Rs, the effects of enrichment on variation also need to be studied. Since the majority of statistical tests are basically comparing the size of the effect (the biological “signal”) relative to the amount of variability in the data, the biological effects may be hidden by a large variation in an experiment. However, in contrast to the comparison of group means, only a few studies have aimed at the influence of enrichment on the variation (Eskola et al., 1999; Gärtnner, 1999; Nevalainen, 1999; Mering, 2001; Tsai et al., 2002; Tsai et al., 2003b).

In the present study the response to enrichment was measured on the basis of breeding performance (for comparing the well-being status between different housing conditions), physiological variables (such as haematological data, body weight gain and relative organ weights), and behavioural tests (Open Field, Food Drive and Elevated Plus Maze) after long-term enrichment.

Non-enriched and enriched groups were always provided with the same cage type to avoid the influence of floor space on agonistic behaviour, which can lead to different inter-individual distances.

Material and methods
Housing: Two types of enrichment were chosen for the present study to evaluate the effects of enrichment. They contained: 1) a nest box, a wooden climbing bar and nest material according to Scharmann 1993 (E1) and 2) horizontal and vertical dividers, modified from Haemisch and Gärtnner 1994 (E2). The purpose of the former was to meet the nest building and climbing behaviour of mice; the later was to offer the animals a burrowing system (see fig. 1 and 2).

Both control (non-enriched, NE) and test groups (enriched, E1 or E2) of each experiment were always provided the same cage size, as cage size can influence the agonistic behaviour and lead to different inter-individual distances.

Experiment 1: Following 4 weeks of adaptation 60 DBA/2 breeding pairs were randomly divided into three rack systems: a ventilated cabinet, a normal open rack and an individually ventilated cage rack (IVC rack) with enriched or non-enriched type II elongated Makrolon cages, half for each housing. Reproduction performance was recorded from 10 to 40 weeks of age for understanding the influence of enrichment (E1) on breeding performance (detail see Tsai et al., 2003a).

Experiment 2: AJ, BALB/c, C57BL/6J and DBA/2 were used for the subsequent experiments, in a total number of 240 (half for each sex). Animals at 3 weeks of age were marked and
assigned randomly to non-enriched (NE) or enriched (E1 or E2) type III Makrolon cages with equal numbers of cages in same-sex groups of four. Behavioural tests (Open Field, Food Drive and Elevated Plus Maze) were performed at 9, 10 and 11 weeks of age, respectively. At 14 weeks of age blood samples were collected for haematological analysis. The final body weights and organ weights were measured following euthanasia at 15 weeks of age to determine whether there are strain differences in the reaction to enrichment (detail see Tsai, 2002; Tsai et al., 2002 and Tsai et al., 2003b).

Statistic: The mean values were compared using factorial analysis of variance, followed by the Scheffé test (significance level 5%). To achieve independence from mean values, the coefficients of variation (SD/mean value, CV) were used instead of the variance (SD²) or mean absolute deviation (MAD) to compare the variation between the non-enriched and enriched groups. As the CVs were not distributed normally, the CV of each variable was compared using the Wilcoxon signed rank test (nonparametric pair t-test).

Results

Breeding performance
E1 housing (according to Scharmann 1993) did not improve the reproduction, but the variation of breeding performance increased due to enrichment (see tab. 1).

Physiological traits and behavioural tests
The effects of enrichment designs (E1 and E2) are not consistent, but vary according to the variables studied.
1. E1 housing had significant effects on Elevated Plus Maze performance, while significant differences were found in Open Field and Food Drive tests and in relative organ weights (adrenal, kidney, spleen and liver) due to E2 housing (detail see Tsai, 2002; Tsai et al., 2002 and Tsai et al., 2003b).
2. Strains reacted differently to enrichment (E1 and E2, detail see Tsai, 2002; Tsai et al., 2002 and Tsai et al., 2003b).
3. In comparison with NE groups there was a tendency towards an increased CV in enriched groups, especially in physiological traits and in Open Field and Food Drive tests (see tab. 2).

Conclusion

Our data showed that:
1. Environmental enrichment will not automatically improve well-being.
2. Enrichment may affect experimental results and can cause higher coefficients of variation (CV). Such influences were strain- and test-dependent.
3. The effects of enrichment on physiological traits are more focused on the variance than on the mean values, while enrichment significantly affected behavioural performance (group means and variations).

This indicates that the effects of enrichment designs vary according to strain and the variable studied. Furthermore reduction and refinement may conflict with each other. Thus, it will be necessary to evaluate the effects of environmental enrichment on variation, before an enrichment design is introduced into an experiment.
Tab. 1: Breeding performance of different housing conditions

<table>
<thead>
<tr>
<th></th>
<th>NE Mean</th>
<th>CV %</th>
<th>E Mean</th>
<th>CV %</th>
<th>Housing difference p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of litter/dam</td>
<td>5.44</td>
<td>37.9</td>
<td>4.15</td>
<td>57.7</td>
<td>0.0011s</td>
</tr>
<tr>
<td>Total No. of pups born/dam</td>
<td>24.9</td>
<td>51.5</td>
<td>15.9</td>
<td>77.1</td>
<td>0.0176s</td>
</tr>
</tbody>
</table>

CV = Coefficient of variation (SD/mean, %)
NE/E = 27 in non-enriched (NE) and 26 in enriched groups (E)
s = significant difference (p<0.1)

Tab. 2: The comparison of CVs between NE and E1/E2 groups

<table>
<thead>
<tr>
<th>Strain</th>
<th>Physiological traits</th>
<th>Open Field and Food Drive</th>
<th>Elevated Plus Maze</th>
<th>Breeding performance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE vs. E1</td>
<td>NE vs. E2</td>
<td>NE vs. E1</td>
<td>NE vs. E2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBA/2</td>
<td></td>
<td>14/22</td>
<td>13/22</td>
<td>5/12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3/4</td>
<td>2/4</td>
<td>2/12s</td>
</tr>
<tr>
<td>A/J</td>
<td>14/22</td>
<td>12/22</td>
<td>11/22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/8</td>
<td>4/8</td>
<td>3/12s</td>
<td></td>
</tr>
<tr>
<td>BALB/c</td>
<td></td>
<td>16/22s</td>
<td>16/22s</td>
<td>4/7</td>
</tr>
<tr>
<td>C57BL/6</td>
<td></td>
<td>7/8s</td>
<td>7/8s</td>
<td>6/12</td>
</tr>
<tr>
<td>DBA/2</td>
<td></td>
<td>52/88</td>
<td>54/88</td>
<td>18/28s+</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>20/28s</td>
<td>18/48s</td>
</tr>
</tbody>
</table>

The CVs of each group were pooled and compared using Wilcoxon signed rank test (nonparametric test). Data contained 4 strains of mice (A/J, BALB/c, C57BL/6 and DBA/2, both sexes), except A/J data of Food Drive test. Physiological variables: relative body weight gain, relative organ weights and haematological data. 14/22 means: out of 22 compared variables, enriched groups had higher CV in 14 variables. s: significant difference (p<0.05); s*: significant difference (p<0.01); s**: significant difference (p<0.001). For group means, E1 housing had significant effects on Elevated Plus Maze performance, while significant differences were found in Open Field and Food Drive tests and in relative organ weights due to E2 housing.

References

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