
Impact of environmental enrichment in mice.

1: Effect of housing conditions on body weight, organ weights and haematology in different strains

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Summary

Currently, environmental enrichment is a very common means of improving animal well-being, especially for laboratory animals. Although environmental enrichment seems to be a possible way for improving the well-being of animals, the consideration of housing laboratory animals should not only focus solely on animal well-being, manpower and economics but also on the precision and accuracy of the experimental results. The purpose of the present study was to evaluate the effects of enriched cages (nest box, nesting material, climbing bar) on body weight, haematological data and final organ weights.

BALB/c, C57BL/6 and A/J mice, originated from Harlan Winkelmann, were used for the experiments—16 animals of each strain. Animals at 3 weeks of age were marked and separated randomly to enriched or non-enriched cages, in groups of four, half for each housing condition. Both cages were type III Makrolon cages, only the enriched cages contained a nest box, a wood bar for climbing and nesting material. Animals were kept in a clean animal room under specific pathogen free (SPF) conditions. Body weights were recorded every week. Blood samples were collected at 14 weeks of age (white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), and haematocrit (HCT) were analysed). At 15 weeks of age, the animals were euthanized by CO₂ in their home cages, and final body weight and organ weights (heart, liver, kidney, adrenal, spleen and uterus) were recorded immediately.

Although nearly all the test variables were not affected by environmental enrichment in their mean values, the enriched group showed higher coefficients of variation in many variables, and strain differences of both housing conditions were not consistent. The influences of enrichment were shown to be strain- and test-dependent. Such effects may lead to an increase in the number of animals which is necessary or may change the experimental results, especially when a study, using enriched housing conditions, focuses on strain differences.

Since the same enrichment design can result in different influences, a positive or a negative or no adverse effect, due to the strain and the variables studied, researchers need to collect more information before enrichment designs are introduced into experimental plans.

Keywords Inbred mice; strain differences; environmental enrichment; haematological analysis; organ weight

To assure a high precision of results in animal experiments, high levels of standardization

have been introduced into laboratory animal science over many years, especially in respect to genetics, hygiene and environment, including feeding and housing. This high level of standardization has been largely

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responsible for the great decrease in the number of laboratory animals used in experimental research in many countries over the last 10 years.

Recently environmental enrichment has become widely used as a means of improving animal well-being, especially for laboratory animals. Many researchers have shown that animals, including farm animals, zoo animals and laboratory animals, can maintain natural behaviour when they live in an enriched environment (Chamove 1989a,b, Mellen & Shepherdson 1997, Loveridge 1998, Mench *et al.* 1998, Rapaport 1998, Wood 1998).

Although the results of van de Weerd *et al.* (1997) have concluded that an enriched environment will not effect the results (mean values) of experiments, some other publications have shown that animals are more aggressive (Henderson 1975, Haemisch *et al.* 1994, Barnard *et al.* 1996, Haemisch & Gärtner 1997) or that the variation of data may be higher in an enriched environment (Nevalainen *et al.* 1998, Eskola *et al.* 1999, Gärtner 1999, Tsai & Hackbarth 2000).

Therefore it seemed necessary to collect more information about the effects of enriched housing before it is routinely introduced into experimental design.

In this study we chose three different inbred strains of mice and kept them in enriched or non-enriched cages from 3 weeks to 15 weeks of age. The primary hypothesis of this study was to determine if strain differences of physiological measurements (haematological values, body weights and organ weights) were the same or at least similar under enriched and non-enriched housing conditions, and also to investigate the effects of housing conditions in different strains.

Material and methods

Animals and housing

Animals Female mice of the inbred strains BALB/cOlaHsd, C57BL/6JOlaHsd and A/JOlaHsd (16 animals of each strain) at 3 weeks of age, originated from Harlan Winkelmann (Borchen, Germany), were

marked and separated randomly to enriched or non-enriched cages, with half of the animals for each treatment. Every strain was divided into four cages, with four animals per cage.

Environment The animals were kept in an animal room under SPF conditions at a room temperature of $22 \pm 1^\circ\text{C}$, with $55 \pm 10\%$ relative humidity, a 12/12 h light/dark cycle and a light intensity of 25 ± 10 lux.

Housing Both cages, enriched and non-enriched, were type III Makrolon cages (Scanbur, Køge, Denmark) ($37.5 \text{ cm} \times 21.5 \text{ cm} \times 15 \text{ cm}$), and only the enriched cages contained a nest box ($16 \text{ cm} \times 22 \text{ cm} \times 4.5 \text{ cm}$) which was made out of the bottom of a type II Makrolon cage, a wood bar ($16.5 \text{ cm} \times 8.5 \text{ cm}$) for climbing, and nesting material (cotton nestlets, $5 \text{ cm} \times 5 \text{ cm}$, EBECO Company, Castrop-Rauxel, Germany) (Scharmman 1993). Cages were changed once a week. An enriched cage is shown in Fig 1.

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 One hundred and thirty to 140 g wood shaving was used for bedding (Altromin GmbH, Lage, Germany). Bedding was changed once a week.



Fig 1 Enriched cage

Test methods

Test and sampling order All tests and samplings were done in pairs, one enriched and one non-enriched cage at a time. Test and sampling order were continued until all of the animals were tested or sampled. Tests or sampling of the same variables were completed within one week.

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

Euthanasia All mice were euthanized at 15 weeks of age in their home cages. The cage cover was replaced by a safety glass lid with a hole. CO₂ was introduced into the cage by a tube with a flow of 6 l/min (Hackbarth *et al.* 2000).

Terminal data Following euthanasia the body weight and the weight of organs (heart, liver, kidney, adrenal, spleen and uterus) were recorded. From dissection to weighing, the organs were kept in a wet chamber (Petri dish with wet cotton). Body weights were measured using an electronic balance (Item number: E0D110, Ohaus Corporation, Florham Park, USA), and a more sensitive instrument (ST-200, Denver Instrument Company, USA) was used for organ weights.

Experimental design

Following one week of acclimatization, from 4 weeks of age, body weight, food and water intake were recorded every week until 15 weeks of age. Blood samples were collected at 14 weeks of age (WBC, RBC, HGB, and HCT were analysed). At 15 weeks of age, the animals stayed in their home cage and were

euthanized using CO₂, and body weights and organ weights (heart, liver, kidney, adrenal, spleen and uterus) were recorded immediately.

Statistics

Data were analysed using StatView version 4.5 computer program (Abacus Concepts, Inc., Berkeley, CA, 1994). The entire test items were compared using a two factorial analysis of variance with the factors strain and environment, followed by Scheffé's test (significance level 5%), to analyse the effects of strain, environment and strain-environment interaction (Lee 1999). The coefficients of variation (CV) of each cage were calculated separately, and the average CVs of each item of every strain were pooled and compared using the Friedman test.

Results

Mean values

Although C57BL/6 and BALB/c mice housed in enriched cages often showed a slight increase, and enriched A/J mice showed a slight decrease, during the experiment (Figs 2–4), no significant effects of housing on body weight were found either during the experiment or at the end of the experiment ($F_{1,6} = 0.366$, $P = 0.5675$).

In comparison to non-enriched groups, the haematological data of enriched groups showed a slight decrease in RBC and HCT, and a slight increase in HGB. The data of WBC were not consistent: A/J and C57BL/6 enriched groups showed higher values than non-enriched groups, but BALB/c mice did not. Even though a slight difference between enriched and non-enriched groups was found,

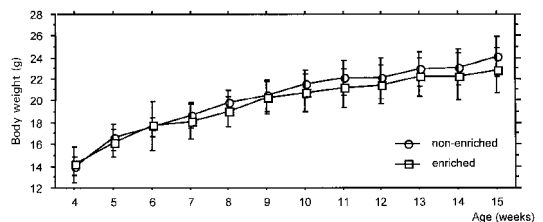


Fig 2 Body weight (g) versus age of A/J mice (mean ± SD)

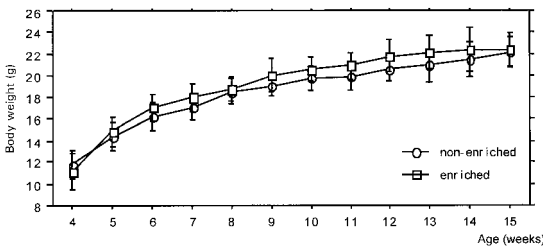


Fig 3 Body weight (g) versus age of C57BL/6 mice (mean ± SD)

no significant housing differences existed in the three inbred strains which were tested (Table 1).

Similar results were found in the data obtained at the end of the study period. Although the reactions to enrichment were not consistent in final body weight, liver weight, kidney weight, heart weight and uterus weight, all enriched groups showed slight increases in spleen weight and slight decreases in adrenal weight in comparison to non-enriched groups. However, none of these data showed significantly different effects of housing on the mean values (Table 2).

Strain differences

Even though there was no significant difference in body weight between enriched and non-enriched groups, strain differences did occur. Both housing conditions showed a significant strain difference on body weight at 4 weeks of age ($F_{2,3} = 20.885, P = 0.0173$ in non-enriched groups; $F_{2,3} = 42.729, P = 0.0062$ in enriched groups), and such significant strain differences were found continuously until 11 weeks of age ($F_{2,3} = 25.016, P = 0.0135$ in non-enriched groups;

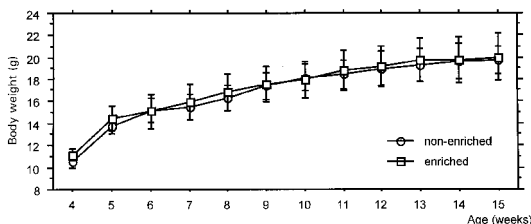


Fig 4 Body weight (g) versus age of BALB/c mice (mean ± SD)

Table 1 The mean value ± SD and coefficient of variation (CV) of haematological analysis

Strain	WBC			RBC			HGB			HCT		
	Standard (CV, %)	Enriched (CV, %)	P	Standard (CV, %)	Enriched (CV, %)	P	Standard (CV, %)	Enriched (CV, %)	P	Standard (CV, %)	Enriched (CV, %)	P
A/J	9.1 ± 1.2 (13.5)	9.9 ± 1.6 (15.9)	$P = 0.0196$	9.2 ± 0.2 (2.6)	9.1 ± 0.4 (4.3)	$P = 0.1006$	9.6 ± 0.2 (2.4)	9.8 ± 0.3 (3.4)	$P = 0.3453$	40.9 ± 1.4 (3.4)	40.6 ± 1.5 (3.7)	$P = 0.0772$
C57BL/6J	11.2 ± 2.9 (26.1)	12.0 ± 2.5 (20.9)	$P = 0.6806$	9.0 ± 0.3 (3.7)	8.9 ± 0.4 (4.1)	$P = 0.1378$	9.1 ± 0.3 (3.2)	9.2 ± 0.3 (3.4)	$P = 0.0524$	40.1 ± 1.2 (2.9)	39.7 ± 1.4 (3.5)	$P = 0.4661$
BALB/c	13.3 ± 3.5 (26.3)	10.8 ± 2.2 (20.7)	$P = 0.7769$	9.9 ± 0.3 (2.8)	9.6 ± 0.4 (4.1)	$P = 0.1006$	10.1 ± 0.05 (0.5)	10.2 ± 0.3 (3.1)	$P = 0.0251$	44.5 ± 1.5 (3.3)	43.7 ± 1.6 (3.8)	$P = 0.0772$
Strain difference			$P = 0.0196$			$P = 0.1006$			$P = 0.0251$			$P = 0.0479$
Housing difference			$P = 0.7769$			$P = 0.3453$			$P = 0.0524$			$P = 0.4661$

WBC = white blood cells, RBC = red blood cells, HGB = haemoglobin, HCT = haematocrit

Table 2 The mean value \pm SD and coefficient of variation (CV) of final body weight (g) and organ weight (g)

Strain	Body weight		Liver weight		Kidney weight		Spleen weight	
	Standard (CV, %)	Enriched (CV, %)	Standard (CV, %)	Enriched (CV, %)	Standard (CV, %)	Enriched (CV, %)	Standard (CV, %)	Enriched (CV, %)
A/J	24.2 \pm 1.6 (6.6)	23.7 \pm 2.1 (8.9)	1.0706 \pm 0.0841 (7.9)	1.0826 \pm 0.0935 (8.7)	0.2857 \pm 0.0150 (5.3)	0.2756 \pm 0.0270 (9.8)	0.0675 \pm 0.0112 (16.5)	0.0798 \pm 0.0305 (38.2)
C57BL/6J	22.1 \pm 1.5 (6.7)	23.0 \pm 1.7 (7.5)	1.0849 \pm 0.1034 (9.4)	1.0664 \pm 0.0953 (8.9)	0.2724 \pm 0.0261 (9.5)	0.2838 \pm 0.0376 (13.2)	0.0764 \pm 0.0090 (11.7)	0.0837 \pm 0.0157 (18.0)
BALB/c	20.3 \pm 1.4 (7.0)	20.5 \pm 2.0 (9.8)	0.9964 \pm 0.0623 (6.2)	1.0096 \pm 0.1429 (14.3)	0.2490 \pm 0.0153 (6.1)	0.2630 \pm 0.0201 (7.8)	0.0836 \pm 0.0076 (9.1)	0.0945 \pm 0.0132 (13.9)
Strain difference	$P = 0.0121$	$P = 0.0513$	$P = 0.3123$	$P = 0.4892$	$P = 0.0952$	$P = 0.1052$	$P = 0.1160$	$P = 0.5593$
Housing difference	$P = 0.5675$		$P = 0.9221$		$P = 0.3710$		$P = 0.1166$	
Strain	Adrenal weight		Heart weight		Uterus weight			
	Standard (CV, %)	Enriched (CV, %)	Standard (CV, %)	Enriched (CV, %)	Standard (CV, %)	Enriched (CV, %)		
A/J	0.0051 \pm 0.0012 (22.6)	0.0049 \pm 0.0004 (8.2)	0.1009 \pm 0.0094 (9.0)	0.0894 \pm 0.0041 (4.5)	0.0619 \pm 0.0120 (19.1)	0.0654 \pm 0.0137 (20.7)		
C57BL/6J	0.0046 \pm 0.0008 (15.1)	0.0043 \pm 0.0007 (16.1)	0.1076 \pm 0.0147 (13.6)	0.1120 \pm 0.0164 (13.7)	0.0787 \pm 0.0245 (30.0)	0.0795 \pm 0.0230 (26.0)		
BALB/c	0.0057 \pm 0.0003 (5.9)	0.0052 \pm 0.0006 (11.3)	0.0929 \pm 0.0094 (10.0)	0.0933 \pm 0.0098 (10.5)	0.0946 \pm 0.0320 (33.6)	0.0877 \pm 0.0076 (9.0)		
Strain difference	$P = 0.1956$	$P = 0.3005$	$P = 0.2906$	$P = 0.0868$	$P = 0.1457$	$P = 0.6412$		
Housing difference	$P = 0.2857$		$P = 0.5968$		$P = 0.9340$			

$F_{2,3} = 15.797$, $P = 0.0255$ in enriched groups). At 12 weeks of age a significant strain difference in body weight was only found in non-enriched groups ($F_{2,3} = 46.017$, $P = 0.0056$), but not in the enriched group ($F_{2,3} = 7.875$, $P = 0.0640$), and this effect was also present at 13, 14 and 15 weeks of age.

As for the body weights, strain differences also occurred in the haematological data, but the effects were not consistent (Table 1). A significant strain difference in WBC was found in the non-enriched groups ($F_{2,3} = 19.116$, $P = 0.0196$), but not in the enriched group ($F_{2,3} = 0.439$, $P = 0.6806$), while no significant strain difference was found in RBC under both housing condition. The data of HGB and HCT had a similar tendency as the data of WBC: a significant strain difference was only found in non-enriched groups ($F_{2,3} = 16.000$, $P = 0.0251$ in HGB, $F_{2,3} = 9.870$, $P = 0.0479$ in HCT).

Both housing conditions showed no significant strain differences at the end of the study, except for the final body weights (Table 2).

However, in all test variables significant effects in the interaction of strain and environment were not found.

Coefficient of variations

Since they are independent from mean values, the CVs (standard deviation [SD]/mean value) within cages were used to compare the variation between the two housing conditions, instead of the variance (SD^2). The data showed that enriched groups had higher CVs for most variables (Tables 1 and 2).

For final body weight, kidney weight, spleen weight, RBC, HGB and HCT, all enriched groups showed a higher CV within cages in comparison to the non-enriched groups, but not all enriched groups had higher CVs of liver weight, heart weight, adrenal weight, uterus weight and WBC (Tables 1 and 2). The median CV of non-enriched groups and enriched groups were 7.9% and 9.0%, and interquartile range of non-enriched groups and enriched groups were 10.3 and 10.4. Use of the Friedman test to compare the pooled CVs of the two

housing conditions showed a significant difference ($df = 1$, $S = 180.5$, $P < 0.001$).

Since the significant effects of housing might be increased due to the correlation between RBC, HGB and HCT or the correlation between body weight, kidney weight and liver weight, the pooled CVs of body weight, spleen weight, adrenal weight, uterus weight, WBC and RBC under the two housing conditions were recalculated using the Friedman test. The housing difference was decreased ($df = 1$, $S = 32$, $P = 0.0626$).

Overall in this study, the mean values of body weights, organ weights and haematological analysis were not significantly affected by environmental enrichment. The effect of strain and the CVs varied between the different variables and between different strains.

Discussion

This present experiment found that the mean body weights or organ weights of enriched animals were slightly changed, heavier or lighter (strain-dependent), and none of these variables showed significant effects from housing differences. Some previous studies have reported that enriched mice had significant heavier or lighter body weights or organ weights on mean values in comparison to non-enriched animals (Thiessen *et al.* 1962, Torre & Cejka 1968, Denenberg *et al.* 1969, Henderson 1970, Manosevitz 1970, Manosevitz & Joel 1973, Manosevitz & Pryor 1975, Clark & Galef 1980, Hara *et al.* 1981, Warren *et al.* 1982, Holson 1986, Peters & Festing 1990, Waston 1993, Haemisch *et al.* 1994, Klein *et al.* 1994, Barnard *et al.* 1996, van de Weerd *et al.* 1997, Nevison *et al.* 1999).

This variation in results suggests that the effects of enrichment may all be influenced by the type of enrichment, the duration of experiments, and the sex and the strain of examined animals.

In comparison to some other enrichment studies, the effects of our enrichment design on mean values were mild, but strain differences were noted. Significant strain differences of non-enriched groups in body weight were present throughout the experiment,

while enriched groups showed significant strain differences at 4 weeks of age, but not after 12 weeks of age. The non-significant decreased body weights of enriched A/J mice and the non-significant increased body weights of enriched C57BL/6 mice may be the cause of this result.

The strain difference in the body weights of enriched groups was slightly decreased with time, while the non-enriched groups showed a similar strain difference during the experiment. This seems to suggest that strain differences on body weight might be influenced by our enrichment in a long-term experiment and that the advancement of such effect is gradual.

The different effects in enriched A/J and C57BL/6 and enriched BALB/c on WBC lead to the different effects of strain differences ($F_{2,3}=19.116$, $P=0.0196$ in the non-enriched groups and $F_{2,3}=0.439$, $P=0.6806$) in the enriched group. Although the mean values of haematological variables in this study are comparable to those of Chvédoff *et al.* (1980), Dahlborn *et al.* (1996) and Eskola *et al.* (1999), housing conditions did not significantly influence the haematological variables or physiological parameters. Due to their high correlation, RBC, HGB and HCT showed a similar effect of enrichment in all tested strains, but the different extent of increasing or decreasing mean values in the three strains also caused a significant strain difference in non-enriched groups and a non-significant strain difference in enriched groups. These results seem to suggest that the reactions to enriched housing condition of the three tested strains on haematological data were not consistent and that such inconsistent reactions also can lead to decreased strain differences in WBC, HGB and HCT.

The results of this experiment seem to suggest that the mild effects on mean values can also lead to changes in different strains and that such effects may change the experimental results in some tests, when a study, using enriched housing conditions, focuses on strain differences.

The results of our study show that environmental enrichment can cause different influences in CVs, and increases or decreases

or no effect, dependent on the strain and the variables studied. In general, enriched groups showed higher CVs for most variables. Similar results can be obtained from data published in previous reports (Thiessen *et al.* 1962, Manosevitz & Pryor 1975, Schapiro & Kessel 1993, Watson 1993, Haemisch & Gärtner 1994, Bergmann *et al.* 1994/95, Eskola *et al.* 1999, Nevison *et al.* 1999). This effect on CV may account for the variations in the effects of environmental enrichment and strains on the variables studied by these research groups.

A higher CV means that the variability of animals may be increased: it can lead to more animals being needed for a given experiment. An overview of the distribution of the comparison between two housing conditions on the number of animal needed in the terminal and the haematological data is shown in Fig 5. The calculation of sample size was based on the present data using JMP 4 power analysis, (to detect a 20% difference in group mean, $P=0.05$, power at 0.90).

Even though the effects of environmental enrichment were decreased, and did not reach a significant difference ($df=1$, $S=32$, $P=0.0626$), when the high correlated variables were not included into the calculations,

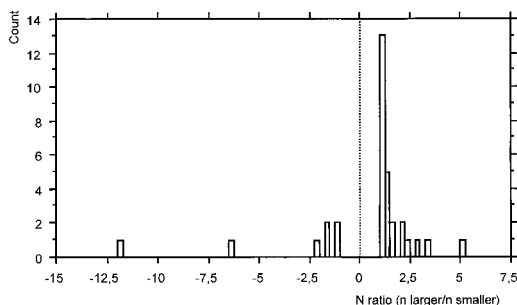


Fig 5 Distribution of the comparison on the number of animals needed in terminal and haematological data. N ratio (n larger / n smaller) were calculated to compare the number of animals needed for enriched and non-enriched groups. A N ration < -1 means that the necessary number of animals in non-enriched groups was higher; a N ratio > 1 means that the necessary number of animals in enriched groups was higher. There is no N ratio between 1 and -1 . The calculation of sample size is based on the present data using JMP 4 power analysis (to detect a 20% difference in group mean, $P=0.05$, power at 0.90)

the P value was close to reaching a significant housing difference. Such a tendency seems to suggest that the chance of having an increased variation due to our enrichment was higher, although for some variables non-enriched groups also had a decreased CV.

The combination of other studies and this present experiment indicates that enrichment designs can cause different influences in CVs, a positive or a negative or no effect, depending on the type of enrichment, the strains, the variables studied and the duration of the experiment. Such effects should be taken into consideration whenever an experimental procedure is planned.

Summarizing the results of this study suggests that values of body weight, haematological variables measures and organ weights were not significantly influenced by our enrichment design, but that enrichment seemed to have effects on the variation and the strain differences. These effects are, however, strain- and test-dependent.

According to these results, enrichment may not have the same or a similar effect for every strain or for every experiment. Researchers need to collect more information before enrichment designs are introduced into experimental procedures.

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