

LONG-TERM BEHAVIOURAL AND PHYSIOLOGICAL EFFECTS OF NESTING MATERIAL AS ENVIRONMENTAL ENRICHMENT FOR LABORATORY MICE

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SUMMARY

Environmental enrichment may improve the quality of life of captive animals by altering the environment of animals so that they are able to perform more natural behaviour. When enrichment is introduced into an animal's environment, it is important to evaluate the effect of the enrichment programme and to assess whether the animal continues to use the enrichment in the long term.

Groups of mice were housed under either standard or enriched conditions for several weeks. Nesting material which was highly preferred in previous studies was used as enrichment. During the period of differential housing several behavioural parameters (behavioural tests and handling) and physiological parameters (urine and plasma corticosterone, food- and water intake, body- and adrenal weight) were monitored to determine the impact of environmental enrichment. Observations were made to determine whether or not the mice continued to use the enrichment.

The results indicated that throughout the study all mice used the nesting material to build nests and that mice from enriched conditions weighed more than mice housed under standard conditions, although the latter consumed more food. No major differences for behavioural and physiological parameters were found between the groups of mice housed under different conditions. Therefore it is not likely that supply of nesting material will jeopardise the outcome of experiments.

INTRODUCTION

Improvement of the quality of life for laboratory animals has received a lot of attention in recent years. Environmental enrichment may ameliorate some of the problems caused by containment (such as the occurrence of abnormal behaviour) by altering the environment of animals in such a way that they are able to perform more of the behaviour which is within the range of the animal's species-specific repertoire (Beaver 1989; Chamove 1989a; Scharmann 1991). Captive environments may be chronically stressful to animals if there is no or only limited opportunity for active behavioural responses as a means of coping with aversive stimulation (Carlstead et al 1993). Well designed housing systems allow for effective coping behaviour, which may enhance welfare (Wechsler 1995).

When enrichment is introduced into an animal's environment, it is important to evaluate the enrichment programme used. It should be assessed whether or not the animal uses the enrichment, and whether the objectives are achieved. It is also important to assess whether the animal continues to use the enrichment in the long-term (Bloomsmit et al 1991; Chamove 1989a; Van de Weerd & Baumans 1995).

Several effects of enrichment on the behaviour of laboratory rats (e.g. Renner & Rosenzweig 1986b; Rose et al 1985; Widman & Rosellini 1990) and mice (e.g. Manosevitz 1970; Manosevitz & Joel 1973; Manosevitz & Montemayor 1972; Manosevitz et al 1968) have been described. In an earlier study, Van de Weerd et al (1994) showed that mice from enriched housing conditions differed from mice kept in standard environments when submitted to several behavioural tests.

Enrichment may not only influence behaviour of the animals but can also have an effect on physiological parameters (Haemisch et al 1994; Kingston & Hoffman-Goetz 1996; Whary et al 1993). A variety of behavioural and physiological parameters can be monitored to determine the impact of environmental enrichment (Markowitz & Line 1990). A widely recognised indicator of stress or anxiety is the measurement of the level of adrenal activity. Altered hormonal levels may indicate that an animal is experiencing aversive stimuli or is having difficulties to cope with a specific situation (Carlstead et al 1993; Broom 1988; Dahlborn et al 1996; Wechsler 1995). Prolonged elevated levels of plasma corticosteroids have adverse effects on the immune system (Riley 1981).

Enrichment may indeed influence corticosteroid levels as was found by Hull et al (1976): gerbils from enriched environments had lower cortisol levels than gerbils from non-enriched groups. Methods to obtain the blood samples needed for the assessment of plasma corticosteroid levels, are regarded as invasive and therefore stressful for the animal. An alternative method for mice which often have

to be killed in order to get enough blood, may be the measurement of corticosterone in urine as described by Dahlborn et al (1996). The use of urinary cortisol as a non invasive, stress free method for assessing changes in adrenal activity has been used in humans (Brantley et al 1988; Kiecolt-Glaser et al 1984). Carlstead et al (1992, 1993) validated the method for felids and used it for the assessment of stress in laboratory cats. Dahlborn et al (1996) found differences in corticosterone levels between mice from three different housing conditions by using the urine method.

In the present study groups of mice were housed under either standard or enriched conditions for several weeks and during this period behavioural and physiological parameters were monitored based on the study by Dahlborn et al (1996). The behavioural tests used were an open field test with objects and an aluminium foil test (a combination of an emergence test and exploration test) to study the reactions of the mice in these novel environments with unfamiliar objects. Behavioural differences between mice from enriched and standard conditions were detected with these tests in previous studies (Dahlborn et al 1996; Van de Weerd et al 1994). The physiological parameters monitored in this study were, besides corticosterone measurements in urine and plasma, food- and water intake, body weight and adrenal weight. These variables have been reported to differ between animals from enriched and standard conditions (Hull et al 1976; Thiessen et al 1962; Van de Weerd et al 1994) and also between strains (Van de Weerd et al 1994).

Handling is a common procedure in laboratory animal husbandry and effects of handling on rodent behaviour have been described. In general, handled animals became less emotional or fearful (Denenberg & Morton 1962; Escorihuela et al 1995; Mazurski 1994). Little is known about the influence of enrichment on the reaction to handling of mice. Therefore, a score was given during weekly cage cleaning to observe if mice from enriched or standard housing conditions show different behavioural responses to handling.

The enrichment used in this study was based on the outcome of several previous experiments investigating the preferences of mice for different enrichment objects and materials (Van de Weerd et al submitted/a; accepted/a/b). In the present experiment the highly preferred nesting material from these previous studies was used as enrichment. Two different strains of mice were used to detect possible strain differences.

Choice tests mostly establish preferences of individual animals over short time periods and it is thus not known if these preferences are beneficial to an animal's welfare in the longer term (Fraser 1996), or what the effects are in groups of animals. In the present study the enrichment was given to mice in groups for a longer period of time to study effects on behaviour and physiology and to observe

if mice continue to use the enrichment.

ANIMALS AND METHODS

Animals

Thirty-six male and 36 female mice of two inbred strains (C57BL/6NCrIBR and BALB/cAnNCrIBR) were used. At the start of the experiment (week 0) they were three weeks of age. The whole experiment lasted 11 weeks. The mice were housed in a room with conventional hygiene and controlled photo-period (lights on 6.00-18.00 h, white light 225 lux), relative humidity (55-75 %) and temperature (22-24 °C). The mice were housed in groups of three (same sex and strain) in wire topped Macrolon type II cages (375 cm², UNO Roestvaststaal, Zevenaar, The Netherlands) provided with 50 g of sawdust (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany). Tap water and food-pellets (RMH-B, Hope Farms, Woerden, The Netherlands) were provided ad libitum.

Groups of three mice were composed on the basis of body weight of the mice at three weeks of age, so that groups had comparable mean body weights. The groups were then randomly allocated to either standard housing conditions (36 mice, 12 groups), the cages only provided with sawdust bedding (Lignocel 3/4) or enriched housing conditions. Enriched cages were provided with sawdust bedding as well as nesting material (3 Kleenex tissues, Kimberly-Clark Corporation[®], EC). The tissues were renewed weekly with cage cleaning. The animals were individually marked on the tail by a colored water proof marker.

PHYSIOLOGICAL PARAMETERS

Body weight, food and water intake

Each week throughout the study, the mice were weighed individually and food and water intake were measured per group.

Urine sampling

Every two weeks urine samples for corticosterone and creatinine analysis were collected (method described by Dahlborn et al 1996). In the morning (between 9.00 and 10.00 h) the mice were placed individually in an empty Macrolon type I cage (204 cm²) until they urinated, but no longer than 50 minutes. Urine was then collected with a syringe and stored in polypropylene tubes at -20 °C. Urine of males and females was collected alternating weekly, thus urine of males was collected in weeks 2, 4, 6, 8 and 10 of the experiment (at the ages of 5, 7, 9, 11 and 13 weeks) and of females in weeks 3, 5, 7, 9 and 11 of the experiment (at the

ages of 6, 8, 10, 12 and 14 weeks).

BEHAVIOURAL PARAMETERS

Handling

During weekly cage cleaning the mark on the tail of the mice had to be renewed. This was done by putting the mouse on the table and holding it by its tail with one hand, while with the other hand the mark was redone. A score was given for the behaviour during this handling procedure. Because individual housing during urine collection might influence behaviour during tail marking afterwards, two handling scores were given: one week a score for the behaviour during normal handling (no urine collected) and the next week a score for the behaviour during handling after urine was collected.

The following scores were used:

- 1 = mouse is sitting tranquil, does not move.
- 2 = mouse moves a little with head or body.
- 3 = mouse is 'walking' with front paws.
- 4 = mouse is 'running' with front paws, sometimes turns around, pulling to get away.
- 5 = same as 4 but the mouse is also squeaking.
- 6 = same as 4 but mouse is also turning around and wrestling frantic to get free.
- 7 = same as 6 but the mouse is also biting or tries to bite.

Open field test with objects

In the 6th week of the experiment (age of the mice: 9 weeks) the mice were subjected to an open field test with two objects (see also Dahlborn et al 1996). These objects were added to the open field to observe if mice from enriched or standard housing conditions would react differently towards these objects. The circular open field (\varnothing 90 cm) was surrounded by a gray PVC wall (height 50 cm). The floor was divided into one inner and one outer circle and in total the area was divided into 12 more or less equal areas. Two clear perspex, V-shaped objects with holes (cheese slice, IMS, Cheshire, UK), were placed (upside down) on the radius where it crossed the inner circle. During testing light intensity was 60 lux (floor level). At the start of a test each mouse was placed in the same area (near an object) and observed during 10 min. Testing (per strain) was conducted on two consecutive days. The first day mice from standard conditions were tested, the next day mice from enriched conditions. Testing started at 9.30 h. Between two tests, the apparatus was cleaned with alcohol. A video system was used to record each test, so that the experimenter did not need to be present in the testing room. Afterwards the behaviour of the animals was scored from video tape using a

behavioural observation software package (The Observer v 2.0, Noldus bv Wageningen, The Netherlands).

Based on the study by Dahlborn et al (1996), only behavioural elements which previously indicated differences between animals from different housing conditions were scored. The following behavioural elements were scored.

Duration (in s) of:

locomotion	=	walking.
immobility	=	sitting idle, not walking, includes freezing.
interaction with object	=	sniffing, gnawing object, walking through a hole in object, sitting under or rearing against object.
sitting on object	=	climbing on and sitting on object or walking over it (four feet from the ground).

After the test the fecal boli were counted.

Aluminium foil test

In the 9th week of the experiment (age of the mice: 12 weeks) the mice were subjected to an aluminium foil test (see also Dahlborn et al 1996). The test consisted of a Macrolon type I cage with an opening (\varnothing 4 cm) in one of the narrow sides. The floor was covered with 25 g of sawdust (Lignocel 3/4). A piece of aluminium foil (10.5 x 14 cm) was placed opposite to the opening in the cage. The small cage was placed in a Macrolon type III cage (840 cm²) containing 75 g of sawdust (Lignocel 3/4). The mouse was placed in the small cage between the opening and the aluminium foil, with its head towards one of the long sides. During testing light intensity was 80 lux (cage level). The observation time was 10 min. Testing (per strain) was conducted on two consecutive days, the first day mice from standard conditions were tested, the next day mice from enriched conditions. Testing started at 9.30 h. Between two tests sawdust and aluminium foil were renewed and the cages were cleaned with alcohol. This test was also recorded on video tape, so that the experimenter did not need to be present in the testing room. Afterwards the behaviour of the animals was scored from video tape using a behavioural observation software package (The Observer v 2.0).

Based on the study by Dahlborn et al (1996), only behavioural elements which previously indicated differences between animals from different housing conditions were scored. The following behavioural elements were scored.

Duration (in s) of:

latency out	=	time to leave the small cage.
latency in	=	time to return for the first time into the small cage.
latency digging	=	time to start digging for the first time.
interaction foil	=	all interactions with the foil, including gnawing, on or under

		foil, sniffing foil.
digging	=	digging in the sawdust of the small or large cage.
grooming	=	licking fur, snout, genitals or tail.
climbing	=	climbing on the walls of the small cage with four feet from the ground

EUTHANASIA

Collection of blood and adrenals.

At the end of the experiment mice of one strain and housing condition were euthanised on the same day between 10.00 and 11.30 h. A cage with a group of mice was transported to a room next to the animal room, where all mice of this group were killed at the same time by 3 technicians, in order to rule out order effects on plasma corticosterone concentrations. Mice were decapitated (using scissors) within a few seconds after fixation, blood was collected in ice-cooled 2 ml tubes containing K2-EDTA (2.0 mg/ml, Greiner, Alphen a/d Rijn, The Netherlands). Adrenals of male mice were removed, as soon as the blood was collected. Adrenals were put into pre-weighed sheets of aluminium foil and weighed afterwards.

ANALYSES

Urine corticosterone and creatinine

Urinary creatinine concentrations were determined with the use of a commercial test combination (Creatinine, MA-KIT 10 ROCHE, Roche Diagnostics) on a COBAS-BIO auto-analyser (Hoffmann-La Roche BV, Mijdrecht, The Netherlands).

For corticosterone analyses 100 μ l of urine was extracted with 200 μ l of dichlormethan. Then, 5 μ l of the dichlormethan phase was transferred in duplicates and analysed according to the method described by Westerhof et al (1992).

Plasma corticosterone

Blood was separated by centrifugation (3000 rpm for 10 min and 10000 rpm for 2 min, at 20 °C) and the plasma stored at -20 °C until assayed. Total corticosterone plasma concentration was measured in duplicate using a double antibody 125I Rats & Mice RIA-kit (ICN Biomedicals, Zoetermeer, The Netherlands).

Statistical analyses

The results of the tests were analysed using SPSS/Windows 6.0 statistical computer programme (SPSS Inc. Chicago, USA).

Food and water intake were analysed for possible differences between

strains, sexes and housing conditions and also time effects using repeated measures MANOVA. Body weight was analysed in a similar way, taking possible group effects into account as well.

Urine corticosterone, the ratio of corticosterone and creatinine and plasma corticosterone appeared to be not normally distributed, therefore they were logarithmically transformed. Urine corticosterone, the ratio (with time before urination as covariable) and plasma corticosterone were analysed for differences between strains, sexes and housing conditions using ANOVA, taking into account possible group effects. Data of two-weekly urine samples were analysed separately, so five analyses on urine corticosterone and ratio were performed.

Adrenal weights of the males were analysed for strain and housing condition effects using ANOVA. The data from both behavioural tests were analysed with MANOVA, for differences between strains, sexes and housing conditions. Fecal boli produced in the open field were analysed with a Mann-Whitney U test. Weekly handling scores (normal and after urine sampling) were analysed using a non-parametric test (Mann-Whitney U) for housing effects.

When multiple comparisons were made, a correction of the P-value with Bonferroni's adaptation was made. The level of statistical significance was pre-set a $P < 0.05$.

RESULTS

Body weight, food and water intake

Figure 1 shows the body weights of the mice during the experiment. There was an overall significant difference between housing conditions. Mice from enriched housing conditions weighed more than mice from standard housing conditions ($P < 0.05$). Overall, BALB/c mice were heavier than C57BL mice ($P < 0.01$) and males weighed significantly more than females ($P < 0.01$). A significant time effect was found as well ($P < 0.01$), reflecting an increasing weight gain in the course of time.

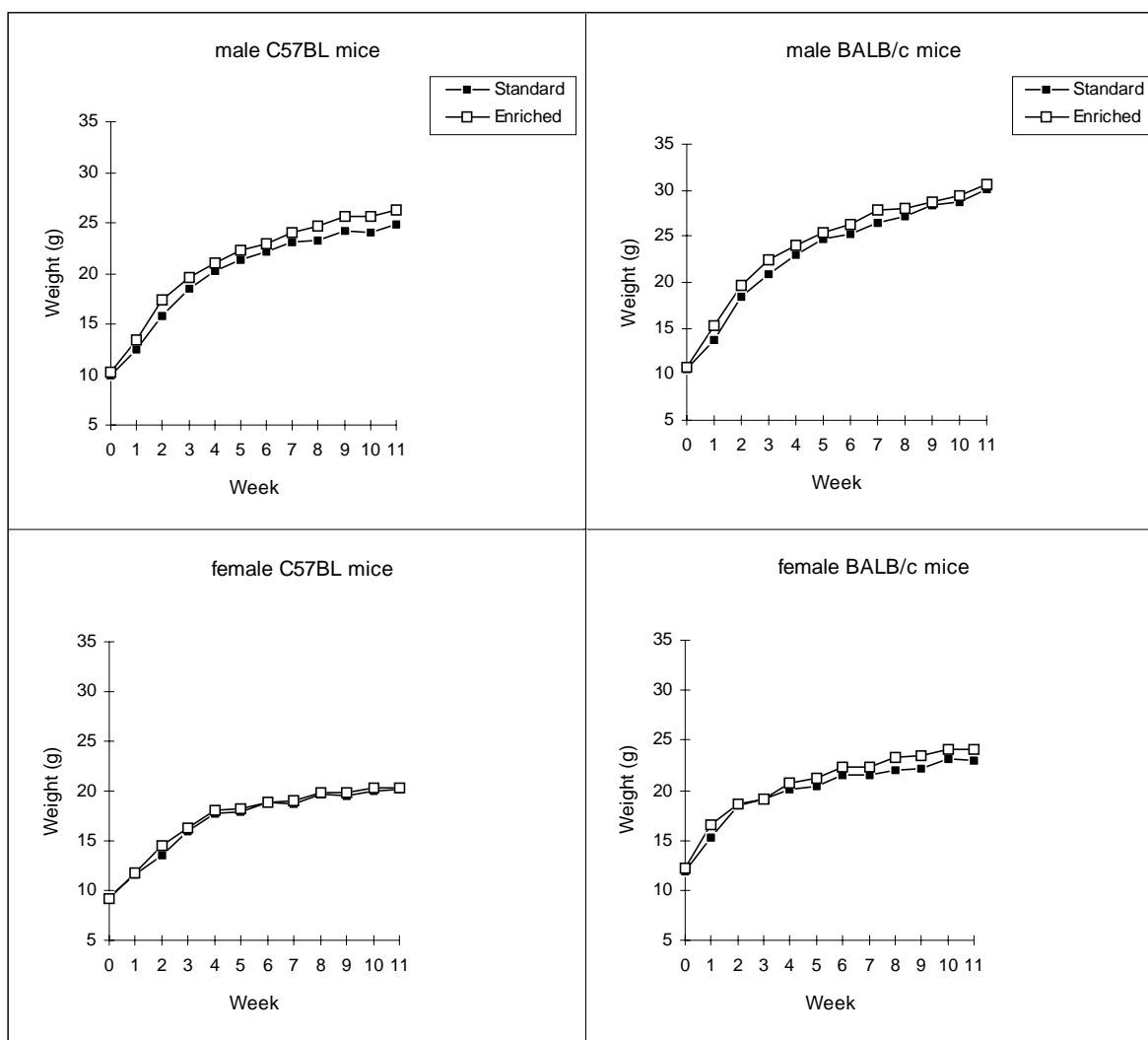


Figure 1 Mean body weight of female and male mice from two strains ($N=72$) during 11 weeks of housing in either standard cages or cages enriched with tissues as nesting material.

Figure 2 shows food intake of the mice during the experiment. There was an overall significant difference between housing conditions, mice from enriched housing conditions consuming less food than mice from standard housing conditions ($P<0.01$). Furthermore, BALB/c mice ate more than C57BL mice ($P<0.01$) and males consumed more food than females ($P<0.01$). Water intake did not differ between the housing conditions (data not shown). Differences between the sexes however, were found ($P<0.01$), males consumed more water than females.

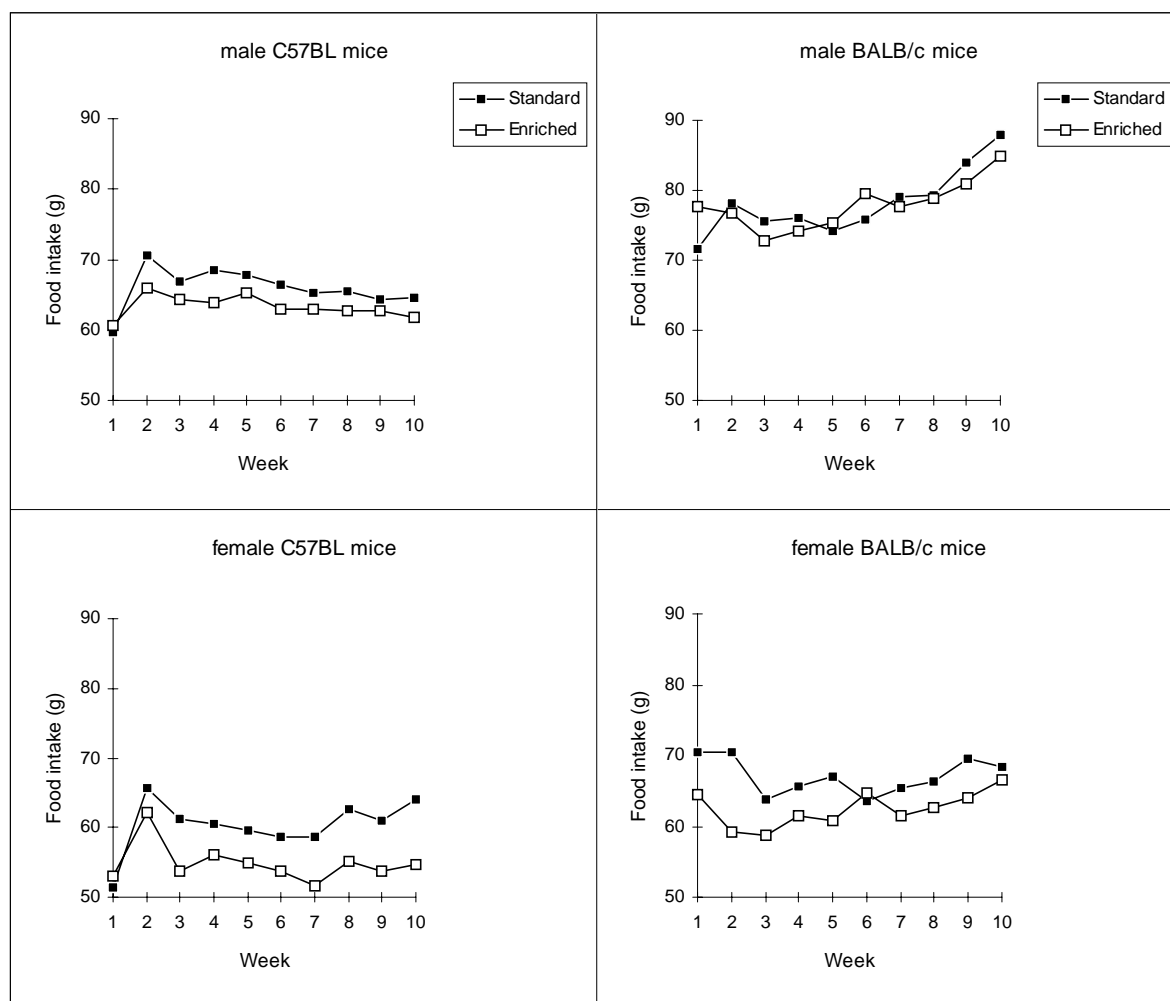


Figure 2 Mean food intake of groups female and male mice from two strains (N=72) during 11 weeks of housing in either standard cages or cages enriched with tissues as nesting material.

Urine and plasma corticosterone

Not all urine samples could be analysed, because mice did not always produce enough urine, but in general, 5-9 samples per group of mice could be used each week.

Differences in urine corticosterone concentrations (Table 1) between housing conditions were found for BALB/c mice only in the fifth sample, BALB/c mice from the enriched conditions had a higher corticosterone level ($P < 0.05$). Further differences between housing conditions were not found.

Significant differences between the strains were found in the first, third and fifth sample (all $P < 0.05$), in general BALB/c mice had higher levels. Significant differences between the sexes were found in all samples (all $P < 0.01$), except for the first sample of the C57BL mice. Females had much higher levels as males.

Table 1 Urine corticosterone values (mean and SD)

	Sample	Wk	Urine cortico (nmol/l)		Ratio cr/co (nmol/mmol)	
			Standard	Enriched	Standard	Enriched
BALB/c males (n=18)	1	2	42.4 (28.4)	36.0 (22.3)	7.7 (4.6)	7.8 (4.8)
	2	4	46.0 (30.8)	52.6 (31.4)	8.0 (5.8)	9.1 (4.4)
	3	6	21.8 (0.8)	40.4 (14.6)	4.0 (0.3)	6.5 (2.3)
	4	8	33.2 (4.4)	59.6 (31.3)	5.1 (0.6)	11.5 (8.1)
	5	10	54.5 (13.5) a	76.6 (17.6) b	10.3 (3.0)	11.2 (3.7)
BALB/c females (n=18)	1	3	163.3 (105.0)	287.9 (109.2)	25.0 (12.6)	50.8 (16.4)
	2	5	191.6 (86.5)	185.6 (73.8)	35.8 (18.0)	31.1 (10.5)
	3	7	277.9 (110.2)	252.4 (97.7)	44.5 (18.2)	44.2 (14.6)
	4	9	213.7 (108.5)	193.4 (90.4)	37.1 (17.2)	38.8 (13.9)
	5	11	198.0 (60.5) a	236.9 (70.3) b	34.6 (11.8)	43.2 (15.5)
C57BL males (n=18)	1	2	133.9 (100.9)	122.4 (56.0)	17.6 (12.1) a	19.4 (6.6) b
	2	4	34.7 (30.3)	37.0 (28.9)	6.4 (5.4)	7.0 (3.3)
	3	6	47.8 (34.4)	24.2 (14.1)	12.0 (11.0)	5.1 (2.1)
	4	8	70.3 (72.6)	24.9 (11.1)	17.3 (19.2)	4.5 (1.6)
	5	10	43.0 (28.1)	28.2 (19.8)	8.2 (5.0)	5.0 (3.2)
C57BL females (n=18)	1	3	141.6 (64.6)	176.2 (84.4)	32.7 (18.3) a	51.6 (42.9) b
	2	5	220.9 (124.8)	120.4 (39.4)	64.9 (18.9)	30.7 (18.1)
	3	7	143.6 (67.9)	146.3 (82.9)	42.8 (35.5)	42.9 (31.7)
	4	9	181.1 (64.0)	153.0 (73.6)	39.1 (9.3)	40.8 (20.5)
	5	11	190.9 (93.8)	198.5 (116.6)	56.2 (42.4)	65.4 (39.9)

a,b indicate a significant difference between the two housing conditions ($P < 0.05$)

Differences between housing conditions in the ratio of urine corticosterone and creatinine (with time to urination as covariable, Table 1) were found for the C57BL mice only in the first sample, C57BL mice from the enriched conditions had a higher corticosterone ratio ($P < 0.05$). Further differences between housing conditions were not found. Significant differences between the strains ($P < 0.05$) were found only in the fifth sample: BALB/c males had higher ratio's than C57BL males and BALB/c females had lower ratio's than C57BL females. Significant differences between the sexes were found in all samples (all $P < 0.01$), except for the first sample of the C57BL mice, and the third sample of the BALB/c mice. Females had higher ratio's as males.

No significant differences between strains, sexes or housing conditions were found for plasma corticosterone. A high degree of between group variation was observed (Table 2).

Table 2 Plasma corticosterone values (ng/ml) in week 11 of the experiment

Standard

Enriched

BALB/c	males	17.9 (14.5)	22.5 (24.9)
	females	35.8 (46.0)	47.4 (35.9)
C57BL	males	12.1 (7.0)	13.4 (15.7)
	females	14.1 (15.8)	51.3 (39.3)

Data given as mean and (SD), N=72

Adrenal weight

No housing effect was found in adrenal weights, but a significant difference between the strains was found ($P < 0.01$). BALB/c mice had larger adrenals (standard 5.9 ± 1.0 mg, enriched 4.9 ± 0.8 mg) than C57BL mice (standard 3.1 ± 1.0 , enriched 3.2 ± 0.6 mg). Because there were significant differences in body weight between mice from standard or enriched housing conditions, the adrenal weights were also analysed with body weight as covariable, but again no differences between the housing conditions were found.

Table 3 Open field test behaviour in week 6 of the experiment

	BALB/c males		BALB/c Females	
	Standard	Enriched	Standard	Enriched
locomotion	74.6 (64.3)	106.0 (72.9)	155.7 (83.3)	55.2 (44.7)
interaction with object	183.6 (173.6)	389.7 (107.8)	254.5 (150.2)	292.5 (232.2)
sitting on object	137.2 (249.5)	10.2 (14.4)	17.8 (28.7)	61.6 (151.0)
immobility	32.8 (51.8)	3.7 (3.7)	19.6 (37.6)	23.0 (25.2)
faeces (no. of boli)	6.7 (1.8)	9.4 (2.9)	4.7 (2.4)	8.0 (2.1)
	C57BL males		C57BL females	
	Standard	Enriched	Standard	Enriched
locomotion	359.7 (24.4)	331.3 (48.3)	346.0 (48.3)	329.3 (33.0)
interaction with object	97.4 (21.5)	115.2 (43.3)	98.3 (16.7)	106.8 (31.2)
sitting on object	20.0 (15.0)	13.3 (11.1)	15.8 (13.7)	11.4 (12.7)
immobility	2.7 (2.0)	7.5 (9.9)	3.5 (2.8)	2.4 (2.2)
faeces (no. of boli)	1.9 (2.1)	0.8 (1.4)	0.4 (0.7)	0.3 (0.7)

Data given as mean duration and (SD), N=72

Behavioural tests

Results of the open field test (Table 3) showed a small, but significant housing effect ($P < 0.05$) in the C57BL strain, when all behavioural elements were analysed together. BALB/c mice from enriched conditions defecated more than BALB/c

mice from the standard conditions ($P < 0.01$). Strain differences were found, BALB/c mice showed more immobility and more interaction with objects, but less locomotion than C57BL mice ($P < 0.01$).

Table 4 Aluminium foil test behaviour in week 9 of the experiment

	BALB/c males		BALB/c Females	
	Standard	Enriched	Standard	Enriched
<i>Mean duration (s):</i>				
interaction with foil	19.1 (25.9)	27.7 (16.4)	30.1 (29.7)	21.8 (19.2)
digging	47.7 (25.9)	28.2 (32.5)	27.5 (21.1)	34.1 (28.1)
grooming	5.8 (4.2)	8.2 (4.8)	12.7 (9.4)	17.8 (9.8)
<i>Latency (s):</i>				
latency out	18.9 (11.6)	40.1 (31.1)	27.8 (15.7)	38.9 (18.8)
latency in	165.1 (135.5)	168.0 (159.6)	163.1 (101.8)	275.8 (185.0)
latency dig	192.4 (46.4)	270.7 (129.5)	228.0 (123.4)	247.4 (136.4)
	C57BL males		C57BL females	
	Standard	Enriched	Standard	Enriched
<i>Mean duration of (s)</i>				
interaction with foil	51.1 (31.9)	57.1 (36.8)	44.1 (25.8)	35.3 (24.8)
digging	47.1 (20.7)	41.4 (20.6)	23.4 (9.2)	34.2 (13.9)
grooming	11.7 (3.5)	17.2 (7.6)	8.4 (6.2)	11.1 (4.2)
<i>Latency (s):</i>				
latency out	13.9 (7.7)	22.4 (17.6)	13.6 (7.9)	12.5 (7.7)
latency in	178.3 (85.2)	240.8 (212.5)	200.5 (88.5)	161.6 (70.5)
latency dig	143.8 (41.9)	178.1 (84.7)	197.7 (79.7)	123.0 (63.5)

Data given as mean and (SD), N=72

The aluminium foil test did not show housing effects (Table 4). Climbing behaviour was not analysed because it occurred only infrequently. Overall, C57BL mice were more active than BALB/c mice ($P < 0.01$). C57BL mice left the small cage sooner than BALB/c mice (latency out, $P < 0.01$) and also started digging sooner (latency to dig, $P < 0.01$). Differences between the sexes were found, in the BALB/c strain, females groomed more than males ($P < 0.01$), C57BL males dug more than females ($P < 0.01$).

Handling

The scoring of the influence of housing conditions on the response to handling did not reveal consistent results. When the first score for normal handling (no urine collection) was given, male mice from both strains housed under enriched conditions had a higher score (both $P < 0.05$), meaning they were more responsive to handling. However, when the second score for the BALB/c females ($P < 0.01$) and the fifth and sixth scores for the C57BL males were given ($P < 0.01$ and 0.05 respectively), the highest responses were for mice from the standard conditions.

Handling after urine collection also yielded inconsistent differences. When the second, third and fifth scores of C57BL males were given, standard housed mice had higher scores (all $P < 0.05$) than enriched housed animals. Similar results were found for the fourth and fifth scores of C57BL females (both $P < 0.01$). The fifth score of the BALB/c males, however, showed an opposite effect ($P < 0.05$).

DISCUSSION

The largest and most consistent effects between mice from enriched or standard housing conditions were found in body weight and food intake. Mice from enriched conditions weighed more, although they consumed less food. An explanation for this effect may be that the nesting material allows the mice to regulate their body temperature. Throughout the study the mice manipulated the nesting material to form nests in which they slept. The nesting material probably provided insulation, which could reduce body heat loss. As a consequence they might have needed less food. Watson (1993) also found a reduced food intake in individually housed mice provided with gauze pads as nesting material, although she did not find differences in body weight. She gave two possible explanations for her findings: mice with nesting material may have used less energy for stereotypic behaviours and/or they were better able to conserve body heat. This latter effect was described by Chvédoff et al (1980) for groups of mice in comparison with mice housed individually or in pairs. Food consumption was declined in mice kept in groups because they slept huddled together. Several other authors have also found that mice from enriched conditions weighed more than mice from standard housing conditions (Dahlborn et al 1996; Henderson 1970a; Manosevitz & Joel 1973; Van de Weerd et al 1994). However, no differences in body weight have also been reported by other authors (Manosevitz 1970; Thiessen et al 1962).

No differences in corticosterone levels in blood plasma were found, suggesting that corticosterone levels were not altered as a consequence of the different housing conditions. These results are in concordance with Dahlborn et al (1996). Haemisch & Gärtner (1994) and Haemisch et al (1994) found elevated plasma corticosterone levels in male mice from enriched conditions. The main

cause for these findings, however, were the high levels of aggression and the changes in social organisation in the mice housed under the enriched conditions. The development of a social hierarchy plays an important role in animal stress (Peng et al 1989). Plasma corticosterone levels of enriched housed mice did show a greater variability than of standard housed mice (see SD in Table 2). This might indicate that enrichment influences social relationships. However, no major differences in levels of aggression were observed between standard and enriched groups of mice.

In concordance with the plasma measurements, corticosterone measurements in the urine showed no consistent housing effects either. Only the fifth urine corticosterone sample of the BALB/c strain and the first corticosterone/creatinine ratio of the C57BL mice revealed a housing effect. Corticosterone was measured on two levels, neither of which showed major housing effects. Plasma corticosterone (free and protein-bound) reflects levels from one time point, whereas (free) corticosterone in the urine reflects levels from a period of time, assembled in the urine. These differences might explain the effects - although small - found in the urine but not in the plasma samples.

No conclusions can be drawn from the differences in the behaviour scores during handling. The handling scores after urine collection were most consistent. They show a trend that, towards the end of experiment, the C57BL mice from enriched cages are more tranquil than standard animals. However, the last score of the BALB/c males showed an opposite effect. Rats which were handled and had enriched housing experience, were better learners and had lower emotionality scores in an open field test (Denenberg & Morton 1962; Escorihuela et al 1995). Thus it can be hypothesised that animals from enriched environments will react less emotional (more tranquil) and get used sooner to handling procedures as performed in this study. The present results however, are not consistent enough to support this assumption.

The behavioural tests did not indicate major differences between mice from the two housing conditions. In the open field test, the enriched BALB/c mice had higher defecation scores than standard housed animals. This finding is difficult to explain and is not in agreement with the results of other authors (Chamove 1989b; Manosevitz 1970; Manosevitz & Joel 1973). Dahlborn et al (1996) performed a similar open field test. The only differences (from 14 behavioural elements measured) they found between mice from standard environments and from cages enriched with only nesting material, was that C57BL mice from environments enriched with nesting material interacted significantly more with the objects than standard housed animals. But most behavioural differences were found in mice from cages, not only enriched with nesting material but also with objects. Dahlborn et al (1996) also performed the aluminium foil test, and did not find differences

between groups enriched with only nesting material and standard groups. This is in concordance with the present results. Other authors, however, did find differences between mice from standard and enriched housing conditions in behavioural tests (Chamove 1989b; Manosevitz 1970; Manosevitz & Montemayor 1972; Manosevitz & Joel 1973; Prior & Sachser 1994/95; Van de Weerd et al 1994), but they always used cages with many enrichments, comparable with the enriched cage with objects Dahlborn et al (1996) described.

In this study, nesting material which was highly preferred in previous studies, was the only source of (continuous) enrichment. Significant differences in physiological and behavioural parameters were found between strains, but not between mice from standard and enriched housing conditions. The only consistent housing effect was reflected by differences in body weight and food intake. In other studies where major effects have been observed, usually different objects were used and often these objects were changed daily, which may cause arousal and even provoke a stressful situation for the animals. In the study of Dahlborn et al (1996), mice enriched with only nesting material, did not differ in behavioural and physiological parameters from mice from standard conditions, but differences were found when mice from environments enriched with nesting material and objects were compared with mice from standard environments.

No detrimental effects of the use of the tissues as nesting material have so far been reported. A screening for contaminants (similar to screenings of bedding materials) did not reveal any toxic substances. Mice provided with nesting material (gauze pads) have been monitored using a battery of hematological and biochemical values (Watson 1993). No differences between test and control animals were found in all these parameters. Also, no lesions in the gastrointestinal tract were found.

In previous studies mice had a very strong preference for nesting material, probably because it can be manipulated for nest building. The provision of nesting material as enrichment might therefore enhance the welfare of mice (Van de Weerd et al accepted/b). Since nesting material does not influence behaviour and physiology of mice in groups to a large extent, it is not likely that supply of nesting material will jeopardise the outcome of experiments. Thus, there seems to be no good reason to deprive laboratory mice from this form of enrichment.

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