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Enriching the metabolic cage: effects on rat physiology and behaviour

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Abstract

Metabolic cages are used for housing rats and mice for up to five days for collection of urine and/or faeces. The small, barren area of the metabolic cage compromises animal welfare as the animals lack a solid floor, shelter, nest material and social contact. We constructed and tested a practically-applicable enrichment device designed to meet behavioural needs for environmental complexity. The influence of this device on the cage preferences and stress levels of the animals was evaluated. A box-shaped enrichment device was designed and implemented in existing metabolic cages. Male Tac:SD rats were housed for five days in an enriched metabolic cage (EMC; n = 12) or a standard metabolic cage (SMC; n = 12), and data were collected on bodyweight, food and water intake, urination and defaecation, as well as urinary corticosterone and creatinine. Moreover, open-field behaviour and cage preferences were assessed. Rats in both groups gained significantly less weight when housed in metabolic cages. Furthermore, SMC rats failed to increase their weight gain after being housed in the metabolic cage. Defaecation was significantly higher in the SMC than in the EMC and so was urinary creatinine. No group differences were found in open-field behaviour. However, in comparing activity before and after housing in the metabolic cage, only SMC animals exhibited significantly lower total activity. In a preference test, a preference for the tunnel connecting the cages in the preference test and a side preference for the left side were found. This side preference was eliminated when the EMC was placed on the right side, whereas the right side was significantly avoided when the EMC was placed on the left side. Based on these results, we conclude that, to some extent, the enrichment device improved the welfare of rats housed in EMC, compared to those in SMC.

Keywords: *animal welfare, behaviour, enrichment, metabolic cage, preference, rat*

Introduction

Metabolic cages are used for rats and mice when collection of urine and/or faeces is needed. Retention time is often 5–6 h, but may be as high as five days. The small and barren circular area with grid floor (small bars), lack of shelters and social stimuli does not comply with the recommendations of the Council of Europe regarding the type and size of the cage floor, the presence of conspecifics and environmental complexity (Council of Europe 1986). According to these recommendations, the minimum enclosure size for rodents weighing up to 600 g is 800 cm^2 during procedures. However, the floor area of the metabolic cage is 420 cm^2. Moreover, it is stated in the associated resolution that rodents should be provided with solid floors with bedding instead of grid floors, special circumstances excepted (Council of Europe 1992). Rats are in continuous contact with the bottom of the cage, and housing rats on a grid floor has adverse effects, such as a higher incidence of sore feet and decubitus (Claassen 1994; Saibaba *et al* 1996; Mering 2000), enhanced gnawing (Kaliste-Korhonen *et al* 1995) and stereotypic pawing (Baenninger 1967). Not surprisingly, preference tests have shown that rats prefer solid floors with bedding to grid floors (Manser *et al* 1995, 1996; Blom *et al* 1996).

Furthermore, rats housed in metabolic cages are deprived of social contact, a resource that has proven very important to rats (Hurst *et al* 1997, 1998; Patterson-Kane *et al* 2001). The resolution on the accommodation and care of laboratory animals (Council of Europe 1997) states that gregarious species should be group housed as long as the groups are stable and harmonious. However, group housing is not possible in the metabolic cage. Finally, the resolution states that "encouragement should be given to break up the interior space of a cage by introducing objects such as platforms, tubes, boxes, etc and attempts should be made to provide environmental enrichment with objects to explore, carry or transform, unless negative effects are observed on welfare or on the intended scientific use". Moreover, both choice tests (van de Weerd *et al* 1996; Townsend 1997; Manser *et al* 1998a; Eskola *et al* 1999) and operant tests

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(Manser *et al* 1998b) confirm that rats prefer a cage containing a shelter. Townsend (1997) demonstrated that rats provided with a shelter were apparently less fearful than rats without a shelter. Thus, it is reasonable to suggest that providing a shelter for rats will enhance the welfare of the animals. Obviously, neither shelter nor companionship are provided when animals are housed in the metabolic cage.

Finally, the aversive environment may influence the physiology and neurochemical balances of the animal to such an extent that research results may be compromised (Perez *et al* 1997). Stress may induce increased defaecation (Perez *et al* 1997; Abe & Saito 1998; Eriksson *et al* 2004) which could confound the measures of faeces related to metabolic studies.

The aim of the present study was to produce and test a practically-applicable device for enriching the metabolic cage. This device should meet — to some extent — the behavioural needs for sheltering and environmental complexity. The influence of this device on the cage preferences and stress levels of the animals was evaluated. However, enriching the metabolic cage constitutes a challenge since this enrichment must not interfere with collection of faeces and urine. Hence, there must be no horizontal surfaces that male rats will mark with urine and no surfaces upon which faeces may adhere. Animals must be single housed. Moreover, urine and faeces must not be contaminated, which means that no form of bedding or nesting materials can be used. Last, but by no means least, the enrichment device should be user-friendly, easily cleaned and economically acceptable.

The experimental testing of the enrichment device consisted of two experiments. The first experiment aimed to assess the influence of the enrichment on open-field activity; a widely-used measure of fear and emotionality in rats. Moreover, physiological parameters, such as food and water intake, were measured. Excretion of faeces was assessed to establish whether stress-induced defaecation was evident in either of the metabolic cage types. Urination was assessed, and the level of creatinine and corticosterone in urine as well as the urinary corticosterone/creatinine ratio were measured and calculated to assess the level of stress in the rats.

The assumption behind the nature of welfare underlying Experiment 1 is that of 'hedonism'. Hedonism is a philosophical approach, stating that welfare is mainly about the presence of positive mental states and absence of negative mental states. Since we are not yet able to see what is going on inside the animal's head, these values cannot be measured directly and, hence, this definition makes the hedonic view more difficult to work with at a practical level. It is, however, reasonable to suggest that the absence of negative mental states, such as pain, stress and frustration, will lead to increased hedonic welfare (Dawkins 1990; Simonsen 1996; Sørensen 2004; Sorensen *et al* 2004). To assess the possible hedonic improvement of animal welfare due to the provision of the enrichment device, the anxiety and potential stress-related changes in physiology in the rats were measured.

The second experiment was designed as a choice test. In this experiment, rats had to choose between an SMC and an EMC. When allowing the animal to choose between two resources — in this case the two cages — the animal will, through its choice, indicate which environment it prefers. The assumptions behind the nature of welfare underlying Experiment 2 are those of 'experience preference satisfaction' (Sandøe 1996; Jensen & Sandøe 1997; Sørensen 2004). According to this view, a more preferred environment results in higher welfare. The most preferred environmental factors can be identified via the use of, for example, a simple choice test, presenting the animal with the opportunity to choose between two resources, assuming that the animal will choose the more preferred resource. It is, however, important to realise that choice tests only give us an idea of the relative properties of the presented choices. If the animal is given the choice between two aversive conditions, it may show a preference for one of the conditions. Nevertheless, the welfare of the animal is still compromised by exposure to the 'preferred' condition. It is, thus, important to provide the animal with reasonable resources to choose from (Duncan 1992; Sørensen 2004). Moreover, combining the measure of preferences with the measures of stress and anxiety will allow more valid conclusions to be drawn.

Materials and methods

Animals and housing

All animal experiments took place at LEO Pharma A/S, Ballerup, Denmark. Male Sprague-Dawley rats, Tac:SD (Taconic, Ejby, Skensved, Denmark) aged four weeks and weighing between 162 and 219 g, on arrival at the animal facility, were used in both experiments. The animals were housed in pairs in plastic cages measuring 42.5 × 26.6 × 18.5 cm (length × width × height) and with a floor area of 800 cm^2 (Tecniplast 1291H, Eurostandard Type III H, Techniplast, Italy) with a raised wire-mesh lid increasing the cage height from 18.5 to 26 cm. Each cage was provided with enrichment, in accordance with Danish legislation, namely aspen bedding (Tapvei, Finland), a cardboard tunnel (150 × 80 mm; length × width) (Lillico Biotechnology, UK) and an aspen wooden block 17 × 17 × 100 mm (Tapvei, Finland). Cages were changed three times a week, and the tunnel and wood block were replaced when necessary. Tap water and pelleted rat feed (Altromin 1324, Brogaarden, Gentofte, Denmark) were provided *ad libitum*. In Experiment 1, the latter was replaced with powdered rat feed (Altromin 1321, Brogaarden, Gentofte, Denmark), when the animals were housed in metabolic cages. All the animals were housed in the same room, and the temperature was 22 (± 2)°C for both the animal and the experimental rooms, with the relative humidity ranging from 40–60% and artificial lighting running between 0600 and 1800h.

The enrichment device

A prototype enrichment device for the standard metabolic cage was designed and manufactured. The enrichment device was a box-shaped construction that could be connected to a standard metabolic cage (Tecniplast number 3701M081; outer diameter, 26 cm; inner diameter at grid floor level, 11.6 cm, height 18 cm). A standard metabolic

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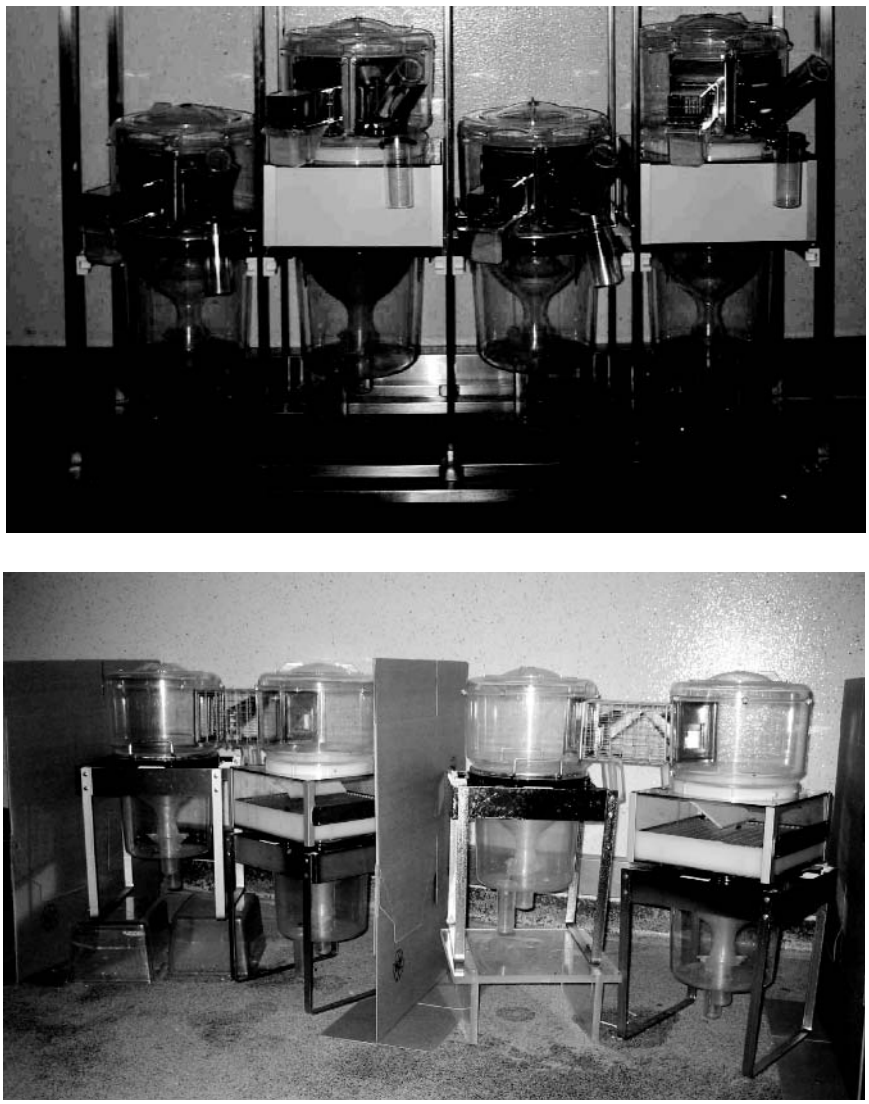
Figure 1



The rack with the EMC and the SMC (Figure 1a, upper image) and the preference test set-up (Figure 1b, lower image). The SMC (Figure 1a, cages 1 and 3 from the left) consists of an upper circular cage part and a lower urine and faeces collector unit. The EMC (cages 2 and 4) is basically an SMC that has been equipped with the enrichment device positioned between the upper cage part and the lower collector unit. The construction of the enrichment device can be seen in Figure 1b (cages 2 and 4 from the left). The upper part of the enrichment device consists of a square cage part with perforated steel floor and a solid ramp for sheltering and for reaching up into the circular part for food and water. The wall next to the area under the ramp is non-transparent. The lower part of the enrichment device is used for connecting to the collector unit.

cage consists of an upper, circular cage part and a lower collector unit. The enrichment device, measuring 27.5 × 27.5 × 13 cm; length × width × height), was inserted between the lower collection part and the upper cage part of the metabolic cage. Hence, the EMC consisted, essentially, of an SMC equipped with the enrichment device. The cage part of the enrichment device was 6.5 cm in height and the bottom part (6.5 cm high) functioned as a funnel, connecting to the collector unit (Figure 1b). The enrichment device had three transparent walls and one non-transparent wall (Figures 1a and 1b). The device had a funnel at the bottom, which was separated from the actual cage by a 2 mm wide perforated steel plate (perforations 8 × 8 mm), specifically designed to ensure adequate drainage of urine. The perforated steel plate was preferred to a grid floor by the constructors as it created a surface more akin to a solid floor than a grid. A 14.5 cm wide, non-transparent, solid ramp, secured at the non-transparent wall, allowed access to the water bottle and feeder chamber in the original circular metabolic cage top. Furthermore, the ramp provided the rat with shelter. Even though the ramp was not horizontal, the intention was that it would still function as an area with a solid floor. To allow rats a proper foothold, the ramp had oblique silicone steps to permit the draining of urine and faeces. The enrichment device increased the floor area from 420 to 756 cm^2 and provided the cage with corners. In the central area, where placement of the metabolic cage top occurred, height increased from 18 cm in an SMC to 24.5 cm

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Table 1 Outcome variables in the open-field test.

Variable	Description
Latency	Time taken for the rat to start moving
Total activity	Measured as number of squares ambulated. A square was considered ambulated when both forepaws were placed within it.
Peripheral*	Peripheral activity measured as number of peripheral squares ambulated
Central*	Number of central squares ambulated
Rearing*	Considered rearing when both forepaws failed to be in contact with the floor
Self grooming	An act of integumentary care, eg grooming
Defaecation	Number of faecal pellets produced during the trial

* Variables were normally distributed.

in the EMC. The ramp and non-transparent wall provided the rat with a shelter that reduced the light intensity from 60 lux in the SMC to 15 lux under the ramp in the EMC.

Experiment 1

Twenty-four rats took part in this segment of the study and on the day of arrival at the animal facility (day 0) each animal was marked on the tail with a permanent marker. The animals were randomly assigned to either the test group (tested in the enriched metabolic cage; EMC) or the control group (tested in the standard metabolic cage; SMC) and placed in pairs in the home cages (each cage housing one animal from the test group and one from the control group). For five days, during the acclimatisation period, each animal was handled once a day for 2 min by the experimenter (stroking and picking up each rat).

Animals were weighed on days 1, 6, 11, 16 and 21. On days 8 and 16 they were tested in an open-field arena. The animals were housed in metabolic cages from days 11 to 16; 12 animals were housed in the EMC and 12 animals were housed in an SMC.

Open field

This test was carried out on days 8 and 16 in an arena measuring 100 × 100 × 40 cm (length × width × height) which was homogeneously illuminated and had white, rubber-lined, steel walls with a white vinyl floor with marked out 20 × 20 cm squares. Sixteen squares were designated as being 'peripheral' (adjacent to a wall) and nine, 'central' (non-adjacent to a wall). A video camera (Panasonic WV BP 330) was placed above the arena and the arena was placed in a room adjacent to the housing room.

One animal was taken at a time from either the home cage (day 8) or one of the metabolic cages (day 16) and transported to the test room. The video camera was set to record, the rat was placed in a corner square of the open field, facing the centre, and the experimenter immediately left the room. Animals were each filmed for 5 min and a number of outcome variables were measured (Table 1). The starting square was altered between animals and after each episode the floor was wiped with a wet cloth and faecal pellets removed.

Physiology in metabolic cages

From days 11 to 16, the animals were housed in metabolic cages; the test group in EMC and the control group in SMC. The test set-up was placed in the room where the animals were normally housed. In order to avoid an influence of cage position on results, the two cage types were distributed evenly in the cage rack (Figure 1a). On day 11, the animals were transferred to the metabolic cages at 0900h and removed on day 16, between 0900 and 1200h as they were tested in the open-field arena. Daily, the following measurements were taken for each cage: decrease in water bottle contents; decrease in feeder chamber contents; amount of urine collected and amount of faeces collected. In addition, eight animals (four from each group), underwent urinalysis whereby urine was frozen at –20°C and corticosterone levels monitored (ng ml^{-1}) via radioimmunoassay (Immuchem™ Corticosterone DA, MP Biomedicals, Solon, Ohio, USA). Further, the creatinine content of the urine (μmol l^{-1}) was also analysed. Creatinine — an end product of muscle metabolism — is believed to be excreted at a relatively constant rate and is, thus, used to correct for the volume of excreted urine. The data on corticosterone levels in urine was therefore analysed both as corticosterone, and as the corticosterone (μmol l^{-1})/creatinine (μmol l^{-1}) ratio. This ratio corrects for differences in urine production rates and hydration status (Brennan *et al* 2000; Fitchett *et al* 2005).

Experiment 2

Twelve rats were used and on arrival at the experimental facility they were marked on the tail with a permanent marker and randomly placed in pairs in the home cages before being given at least 20 days to become acclimatised to their surroundings. All animals were tested twice in a preference test system, one that gave each rat the opportunity to choose between two similar standard metabolic cages, SMC (the first condition; the control condition) and one that presented a choice between an SMC and an EMC (test condition). The timespan between the two tests was either a short delay (varied between 11 and 14 days) or a longer delay (varied between 33 and 35 days) for the individual animal. This time delay was a result of repairs to the test systems and not introduced on purpose. The test set-up consisted of two test cages, connected by a wire-mesh tunnel (11 × 9 × 15 cm; length × width × height). Both cages in a test set-up were provided with equal amounts of food pellets and drinking water. Two test set-ups were in use simultaneously. A cardboard screen between the two systems prevented any visual contact between animals, ensuring there was no difference between the two cages of the individual set-up (Figure 1b). Cages were cleaned with hot water and detergent after each rat. In the standard/enriched preference test, the position of the two cages was switched over once 50% of the animals had been tested. In order to minimise ultrasound from the technical equipment, the time-lapse video and monitor

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were placed behind cardboard boxes, and the monitor was turned off when the tests were being conducted. An infrared lamp, suspended from the ceiling between the two test systems, was used to facilitate recording during the dark period. The positioning of the rat at test start alternated between the two test sessions (left or right cage); this ensured that half of animals started in one cage, while the other half started in the other. Animals were transferred to the test system at 0900h and given 6 h to acclimatise before being recorded for 24 h. At 10 min intervals, the position of the rat in the test system was recorded.

Statistical analysis

The Statistical Analysis System, SAS version 8.2, was used for all statistical analyses (SAS Institute Inc, North Carolina, USA) using a 5% significance level. In the open-field test, the effects of group (EMC or SMC) and time (testing before or after housing for five days in the metabolic cage) were tested on a number of outcome variables (Table 1). Data that were normally distributed were analysed using an analysis of variance, whereas outcome variables that were not normally distributed were analysed by a non-parametric analysis of variance of the rank-transformed outcome variable. Group and time were included as fixed effects. Pairwise comparison of significant variables was carried out using the least-squares means (LSM) procedure.

Food and water intake during the five days of housing in the metabolic cage was analysed using a repeated measures analysis of variance, including group and day as fixed effects. Excretion of urine and faeces was analysed in the same way.

Corticosterone levels, as well as corticosterone/creatinine ratio were analysed using repeated measures analysis of variance on ranked transformed data, as the data were not normally distributed. Creatinine levels in urine were analysed using repeated measures analysis of variance. In all three analyses, first-order autoregressive covariance structure was used for the repeated measures analysis.

The data from the first condition of the preference test (standard metabolic cage tested against standard metabolic cage) were normally distributed and analysed by calculating percent observations in each compartment (left, right and tunnel) and performing an analysis of variance with these percentages as outcome and compartment, test day and start cage as dependent variables. For the second condition of the preference test, which was testing the enrichment device against an SMC, percentage data were not normally distributed. Therefore, an analysis of variance was carried out on ranked data. The initial statistical model for the second conditions included compartment (left, right and tunnel), side of enrichment device (to the left or to the right in the preference set-up) and delay (short or longer) as dependent variables. Moreover, relevant interactions were analysed.

Results

Experiment 1

Open field

Overall, no effect of group was found in open-field behaviour (animals housed in SMC compared to animals housed in EMC). An overall effect of time (before housing compared to after housing in metabolic cages) was found on total activity ($F = 18.24$; $P < 0.0001$), rearing ($F = 18.60$; $P < 0.0001$) and peripheral activity ($F = 18.21$; $P < 0.0001$). Analysing each group of animals (SMC and EMC), separately, demonstrated that animals housed in the EMC showed significantly less rearing after being housed in the EMC, whereas animals housed in SMC showed less activity, less rearing and less peripheral activity after being housed in the SMC (Table 2).

Physiology in metabolic cages

No effect of group was found on weight gain ($F = 14.18$; $P = 0.5912$). Rats in both groups gained significantly less weight when housed in metabolic cages, compared to the week before housing in the metabolic cage (SMC: $t = 6.17$, $P < 0.0001$; EMC: $t = 2.84$, $P = 0.0068$). Both groups gained less weight during and after housing in the metabolic cage (Table 3) compared to periods prior to metabolic cage housing. However, SMC rats failed to increase their weight after being housed in the metabolic cage, whereas EMC rats showed significantly increased weight gain when they were returned to normal housing conditions ($t = -3.58$, $P = 0.0008$). Three animals in the SMC (n = 12) lost weight in the week after being housed in the metabolic cage, resulting in a rather large standard deviation of this variable.

Overall, food consumption was higher during housing in SMC (138.9 [± 10.0] g per five-day housing period compared to EMC (133.94 [± 5.19] g) (chi-square = 6.5636, $P = 0.0104$). However, an effect of day was seen on food intake (Table 4), with food intake being significantly reduced on the first day of housing in the metabolic cage ($P = 0.0003$). Water consumption was also significantly reduced on the first day of housing in the metabolic cage, compared to the other four days ($P < 0.0001$).

Defaecation was significantly higher for animals housed in SMC compared to those in EMC ($P = 0.0022$). No effect of day was found on defaecation.

The volume of urine excreted was affected both by group ($P = 0.0002$) and day ($P = 0.0027$), but a closer analysis revealed that the group effect was due to the fact that it was only in the test group that a significant effect of day was found (Table 4).

The corticosterone level in the collected urine was not affected by housing conditions (SMC versus EMC) or by day (day 1–5). No differences between the individual animals were found and no interactions between housing

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Table 2 Mean (± SD) outcome variables in the open field test.

Housing	Outcome	Before housing in MC	After housing in MC	*t*-value	*P*-value
EMC	Total Activity	135.25 (± 24.49)	117.50 (± 19.40)	1.97	0.0618
	Rearing	31.75 (± 9.63)	20.58 (± 5.78)	3.44	0.0023
	Peripheral	127.42 (± 21.14)	113.08 ± (16.66)	1.84	0.0786
SMC	Total Activity	154.25 (± 20.57)	120.08 (± 17.98)	4.55	0.0003
	Rearing	36.50 (± 13.95)	23.92 (± 7.13)	2.78	0.0109
	Peripheral	143.17 (± 18.80)	111.10 (± 16.31)	4.41	0.0002

EMC: Housed in the metabolic cage; SMC: Housed in the standard metabolic cage; MC: Metabolic cage, either EMC or SMC. All data were normally distributed and analysed using the *t*-test.

Table 3 Pairwise comparisons of mean (± SD) bodyweight gain during four periods of time and under different housing conditions.

Housing	Day 1–6	Day 6–11	Day 11–16#	Day 16-21	*t*-value	*P*-value
EMC (n = 12)	38.20 (± 6.01)	37.34 (± 4.84)			0.32	0.7492
	38.20 (± 6.01)		20.88 (± 5.08)		6.49	< 0.0001
	38.20 (± 6.01)			30.44 (± 9.25)	2.91	0.0057
		37.34 (± 4.84)	20.88 (± 5.08)		6.17	< 0.0001
		37.34 (± 4.84)		30.44 (± 9.25)	2.59	0.0131
			20.88 (± 5.08)	30.44 (± 9.25)	–3.58	0.0008
SMC (n = 12)	36.92 (± 5.22)	40.18 (± 7.02)			–0.60	0.5500
	36.92 (± 5.22)		24.81 (± 4.31)		2.24	0.0304
	36.92 (± 5.22)			20.27 (± 24.64)*	3.08	0.0036
		40.18 (± 7.02)	24.81 (± 4.31)		2.84	0.0068
		40.18 (± 7.02)		20.27 (± 24.64)*	3.68	0.0006
			24.81 (± 4.31)	20.27 (± 24.64)*	0.84	0.4047

Period during which animals were housed in metabolic cage, in the three other periods animals were housed in their home cages.
* Three animals lost weight during this period. All weights were in g.

Table 4 Mean (± SD) food and water consumption and urine and faeces excertion over a five-day period in the metabolic cage

		Day 1*	Day 2#	Day 3#	Day 4#	Day 5#
Food consumption (g)[D]	EMC	25.08 (± 2.04)	26.81 (± 1.51)	27.41 (± 1.64)	27.16 (± 1.60)	27.48 (± 1.38)
	SMC	26.17 (± 2.26)	27.69 (± 2.62)	28.39 (± 2.27)	28.27 (± 2.69)	28.35 (± 1.89)
		Day 1*	Day 2#	Day 3#	Day 4#	Day 5#
Water consumption (ml)[D]	EMC	32.92 (± 3.50)	38.83 (± 2.44)	38.67 (± 3.75)	40.01 (± 2.86)	39.58 (± 3.12)
	SMC	34.08 (± 4.35)	39.50 (± 4.12)	39.75 (3.70)	39.67 (± 5.35)	41.42 (3.00)
		Day 1	Day 2	Day 3	Day 4	Day 5
Urine excreted (ml)[D,G]	EMC	7.58 (± 2.51)*	8.98 (± 2.75) [#,†]	8.83 (± 3.08)[#]	10.11 (± 2.48)[†,§]	10.55 (± 2.39)[§]
	SMC	12.67 (± 2.70)*	13.96 (± 3.15)*	12.97 (3.53)*	13.93 (± 3.53)*	13.61 (± 3.11)*
		Day 1	Day 2	Day 3	Day 4	Day 5
Faeces excreted (g)[D]	EMC	13.98 (± 2.48)	15.43 (± 2.08)	15.51 (± 2.18)	15.27 (± 2.01)	15.02 (± 2.00)
	SMC	18.61 (± 3.66)	17.23 (± 3.44)	17.32 (± 2.61)	17.29 (± 3.12)	18.03 (± 2.99)

D: Significant effect of day; G: Significant effect of group. Days/groups that do not differ are marked with matching symbols.

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Table 5 Median (q1; q3) and percentage time spent in the three different compartments (right-side cage, left-side cage and tunnel) in the preference set-up.

				Pairwise comparisons, *P*-value		
Combination	**Right-side cage (RC)**	**Left-side cage (LC)**	**Tunnel (T)**	**RC:LC**	**RC:T**	**LC:T**
EMC right, short delay	12.50 (7.63; 29.17)	50.69 (4.86; 75.69)	20.14 (16.67; 82.64)	0.5664	0.3593	0.7128
EMC right, long delay	43.06 (15.97; 72.22)	18.28 (9.02; 27.78)	37.96 (9.72; 75.00)	0.39999	0.8539	0.5021
EMC left, short delay	7.64 (6.25; 7.64)	15.97 (11.11; 17.36)	78.01 (75.00; 82.64)	0.0029	< 0.0001	0.0001
EMC left, long delay	23.61 (8.33; 84.72)	74.31 (14.58; 84.72)	2.08 (0.69; 6.94)	0.6413	0.0571	0.0296

Each combination of positioning of the enriched metabolic cage, EMC (on the left side or on the right side in the set-up) and the time delay from the control test (preference set-up with two identical standard metabolic cages) are shown. Data were not normally distributed. ANOVA was performed on rank transformed data and pairwise comparisons were done using the differences of least squared means procedure.

condition and individuals were demonstrated. A power analysis of the study indicated a power of 0.89 even though only a small number of animals were used, therefore a true difference should have been detected with the present data.

The level of creatinine in urine was significantly higher (P = 0.0170) in animals housed in the SMC (6,288.9 [± 675.4]) µmol l^{-1} compared to animals housed in the EMC (5350.6 [± 462.4]).

The corticosterone/creatinine ratio was influenced only by the individual rat. However, the power calculations revealed a power for this variable of 0.77, indicating that with the number of animals used, there would still be a good chance of demonstrating a true difference.

Experiment 2

Preference test

In the first condition, the final statistical model included only compartment (left, right, tunnel) as a dependent variable for which a significant effect was shown (P = 0.0022). Pairwise comparisons of two SMC as a control situation, demonstrated a preference for the tunnel connecting the cages and for the left compartment. The right compartment was favoured significantly less compared to both the left compartment (P = 0.0102) and the tunnel (P = 0.0007). No difference was found when comparing the left compartment and the tunnel (P = 0.3156).

In the second condition of the preference test, the final statistical model included only an interaction between compartment and delay (P = 0.0259). When the delay was short, an interaction between compartment (left, right and tunnel) and the position of the enrichment device was found (P = 0.0326). No overall significant difference was found between time spent in the right, left and tunnel compartment (P = 0.8015). However, the metabolic cage with the enrichment device was placed either to the right or to the left in the experimental set-up. Moreover, for half of the animals, a longer time delay from condition 1 to condition 2 was introduced. The data set was split up and re-analysed (Table 5).

The time spent in each compartment — considering both the time delay and the side in which the enrichment device was placed — was analysed. The avoidance of the right side became clearer when the enrichment device was on the left side; when the enrichment device was placed on the right side (which was avoided in the first condition), no differences were found (Table 5). However, once a month had elapsed since the running of the first condition (long time delay), this right-side avoidance appeared to have disappeared.

Discussion

When housed in metabolic cages, rats provided with an enrichment device ate less, drank less and defaecated less, over the five days. Moreover, the rats in the EMC appeared to urinate less. Although this may have been due to the enrichment device retaining some urine, eg on the ramp or on the larger funnel, it is fair to say that as the rats drank less, they would almost certainly have urinated less. Additionally, food intake was also significantly smaller on the first day in the metabolic cages and, as food and water are noted as complementary resources (ie when food intake goes up, water intake increases, [Hursh 1980]), this relationship is perhaps unsurprising.

A difference in urinary creatinine was demonstrated. The level of creatinine was significantly higher in animals housed in the standard metabolic cage. This corresponds well with findings demonstrating that single housing of rats resulted in significantly higher levels of urinary creatinine compared to group-housed rats (Spangenberg *et al* 2005). Using the corticosterone/creatinine ratio for stress assessment may not be optimal, as urinary creatinine seems to vary with the stress level of the animal and, hence, may not be as constant as often assumed. Another possibility would be to measure the total amount of corticosterone excreted per hour relative to bodyweight which would still take into account the hydration status of the animals as it is the total amount that is being used (Eriksson *et al* 2004).

Even though the rats in the EMC ate less and gained less weight, when housed in the metabolic cage compared to the previous week, their weight gain in the period after being

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housed in the EMC was significantly higher than during housing in the metabolic cage. The rats housed in the SMC, on the other hand, failed to increase/partly normalise their weight gain after being housed in the metabolic cage. The mean weight gain in the SMC rats during and after housing in the metabolic cage did not differ. Three rats from the SMC even lost weight in this last period. It could be hypothesised that the impact on the animals during housing in the metabolic cage was strong enough to persist even after the animals were returned to normal housing. A somewhat similar effect was found in rats housed on a grid floor, which induced an increased systolic blood pressure that remained elevated for at least 12 days after animals had been returned to housing on bedding (Krohn *et al* 2003). This effect, however, was not seen for heart rate which returned to normal once the animals were returned to being housed on bedding.

The results clearly demonstrate that housing both in the EMC and in the SMC is stressful to the animals. These results are consistent with the findings of Eriksson *et al* (2004). However, the results also indicate that housing in EMC decreases the intensity of the stress imposed upon the animals when being housed under poor conditions. While the stressful impact on animals in the SMC is strong enough to persist, having a suppressive effect on weight gain after being returned to the home cage, the EMC animals grew faster in the days after housing in the metabolic cage than they did in the days during housing in the metabolic cage. In other words, the rats housed in the SMC carried the adverse effect on weight gain with them from the metabolic cage to their home cage. The open-field data supported these findings, demonstrating that only the SMC animals, after housing in the SMC, showed less exploration, less rearing and less peripheral activity in the open field than before housing in the SMC. Rats housed in the EMC only showed a decrease in rearing behaviour as a response to housing in the EMC.

In the preference test, the rats, overall, preferred the tunnel connecting the two cages, which, understandably, presented a problem when it came to evaluating the results. Moreover, half of the rats had to wait for four-and-a-half weeks after being run in the first condition before they were tested in the second. From the first condition, it was evident that the right side was, for some reason, less preferred. A possible explanation for this could be that this cage was closer to the door than the left side. However, the results seem to indicate that the factor making the rats avoid the right compartment had disappeared over time, as the preference for not staying on the right side vanished in those rats having had the longer period between the two conditions. An overall conclusion of the preference test is that the rats liked the tunnel the best, but also that the preference for the EMC was strong enough to overcome the avoidance shown towards the right compartment in the first control condition. If the rats were tested in the second condition shortly after the first condition, rats with the enriched cage placed to the right did not show any significant preferences for any of the three compartments — hence the aversiveness of the right side seemed to have disappeared. If the enriched cage was placed to the left, the rats avoided the right compartment even more, choosing the tunnel or — to a lesser extent — the enriched cage.

Therefore, even though we found only minor indications of increased welfare in the enriched metabolic cage, the data on weight gain, urinary creatinine and defaecation, as well as the results of the preference test and the open-field test, indicate that the rats did indeed benefit from the enrichment. However, it is likely that the conditions provided in the metabolic cages are very difficult to improve to an extent where the rats feel significantly more comfortable. On the other hand, though, we remain obligated to do our utmost to improve housing conditions whenever possible and, to this end, the enriched metabolic cage is an important step in the right direction.

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