

Effects of Environmental Enrichment for Mice: Variation in Experimental Results

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This study focused on the effects of different enriched environments for mice in a number of behavioral and physiological parameters in 2 routine laboratory testing procedures: potency testing for tetanus vaccine and stress-induced hyperthermia. The variability in the results was studied by calculating and analyzing mean absolute devi-

ations. Mice from enriched conditions weighed more and consumed more food than mice from standard housing conditions. However, mice from enriched conditions lost more body weight after being housed individually. Other physiological parameters showed no differences. Mice from standard conditions were more active in an open field, suggesting a tendency to overrespond to various stimuli in a testing environment. Mice from enriched environments were more tranquil and easier to handle. The enrichment did not influence the variability in any of the parameters measured, although earlier results and results of other studies suggest that the effects on the variability in results are parameter dependent. When enrichment does not influence variability, there is no reason for not introducing cage enrichment and by doing so contributing to the animals' welfare.

Environmental enrichment, or modifications to the environments of animals to improve their biological functioning (Newberry, 1995), increasingly has been introduced into laboratory animal research facilities. From a welfare point of view, this is a good development, because providing environmental enrichment improves the animal's well-being. Enriched environments release and structure species-specific behavior, meeting more of the behavior needs (Beaver, 1989; Benn, 1995; Chamove, 1989; Fortmeyer, 1982; Newberry, 1995; Van de Weerd, 1996). Especially when the design of the enriched environment takes into account specific behavioral demands of the animals as assessed by means of preference tests (Mench, 1994; Van de Weerd, Van Loo, Van Zutphen, Koolhaas, & Baumans, 1997b), the improved housing conditions may enhance the welfare of the animals. When deprived of the possibility of performing species-specific behavior, animals may show signs of suffering, such as abnormal behavior or other pathology (Jensen & Toates, 1993). Providing environmental enrichment, such as for building nests, gives the animals more control over their environment by structuring it (Beaver, 1989; Benn, 1995; Chamove, 1989; Van de Weerd, Van Loo, Van Zutphen, Koolhaas, & Baumans, 1997b; Van de Weerd, Van Loo, Van Zutphen, Koolhaas, & Baumans, 1998a, 1998b). Increasing the controllability of relevant events may reduce any stress experienced by an animal (Wiepkema & Koolhaas, 1993).

There seems to be some concern, however, as to whether environmental enrichment conflicts with the standardization of experiments. Standardization increases the reproducibility and comparability of experiments. It aims at reducing unwanted variation caused by animal and environmental factors and thus reduces the number of animals needed in experiments (Beynen, Gärtner, & Van Zutphen, 1993; Gärtner, 1999; Van Zutphen, Hedrich, & Van Oortmerssen, 1993). Some researchers fear that "enriched" animals show more variability in their responses to experimental procedures because they show more diverse behavior. In complex environments, animals are responding not just to one stimulus in isolation but to many variable stimuli at once (Appleby, 1997), which may cause increased varia-

tion within subjects (Eskola, Lauhikari, Voipio, Laitinen, & Nevalainen, 1999) or enhance the deviation in experimental data (Gärtner, 1999).

One might argue that because animals can perform more of their species-specific behavior in enriched environments, they may be better able to cope with novel and unexpected changes, thus showing a uniform response (Baumans, 1997; Chamove, 1989; Rose, 1994; Van de Weerd, 1996; Wemelsfelder, 1994). Moreover, animals living in an enriched environment may be less excitable than animals in barren environments because restricting sensory input makes the nervous system more sensitive and more reactive to external stimulation (Grandin, 1989). Also, restrictive rearing appears to produce deficits in learning ability as well as tendencies to overrespond to stimuli in a new environment (Joseph & Gallagher, 1980). Consequently, animals from enriched housing conditions would be expected to be more stable physiologically and psychologically and may, therefore, be considered more refined animal models, ensuring better scientific results (Bayne, 1996; Benn, 1995; Rose, 1994; Spinelli & Markowitz, 1985; Van de Weerd, 1996). They might be more suitable as models for humans, as it is questionable whether keeping animals in nonstimulating, standard environments makes them adequate models (Markowitz & Gavazzi, 1995). If housing conditions do not meet the demands of a particular species, one cannot expect reliable and reproducible results (Fortmeyer, 1982).

If one provides animals in the laboratory with the very best physical and social environmental conditions for their well-being, then one should need to use fewer of them in research experiments or routine tests, and one's results will be accurate and reliable. In these optimal conditions, variation will be greatly reduced (Chance & Russell, 1997; Russell, 1994). This is not a new observation: In the 1950s, Chance (1956, 1957) demonstrated that the size of the variance is related to the nature of the conditions—housing, treatment, and social situation—in which the animal in the laboratory is kept. As many researchers have reported (e.g., Van de Weerd, 1996), this means that enrichment influences not only group means of measured parameters but also the variability of these results. If these expectations are true across a broad range of experiments, then eventually the use of enriched animals in the laboratory may lead to a reduction in the number of animals necessary for obtaining valid experimental results. However, few studies have focused specifically on the variability in results. Moreover, studies use different methods of analysis, such as coefficients of variation per parameter (Gärtner, 1999) or of different groups (Tsai & Hackbarth, 1999) or Solo power analysis (Eskola et al., 1999). In this study we calculated and analyzed mean absolute deviations (MADs) to compare the variability of individual mice, instead of groups of mice, from different environments (meaning a lower N). We calculated the MAD as the (positive) difference between the individual value for one mouse and the mean value from that mouse's group or cage. Zimmerman (1999) used a comparable method for rats, using medians instead of means.

In this study we investigated the effects of enrichment on the results of a number of behavioral and physiological parameters in two routine testing procedures. The selection of the cage enrichments we used (nesting material and nestbox) was based on preference tests conducted by Van de Weerd and colleagues (Van de Weerd et al., 1997b, 1998a, 1998b) and on practical use and previous experience (wire grid floor, plastic tube, gnawing blocks). The first experiment, in which we studied variability in antiserum responses of mice used in potency testing of tetanus vaccine, was carried out at the National Institute of Public Health and the Environment (RIVM; Bilthoven, The Netherlands). A pilot experiment (Van de Weerd, Willems, Hendriksen, & Baumans, 1999) in which the same procedures were used showed no significant effects of the housing conditions on the immune response or the variation in the immune response. In this study, which is a follow-up of the pilot experiment, we used three different housing conditions: tissue, enriched, and superenriched. The provision of a paper tissue is the routine housing condition at the RIVM and, therefore, was included in this study. Previous research into the effects of tissues as enrichment showed no major effects of enrichment on behavioral and physiological parameters (Van de Weerd et al., 1997a). Because the degree and form of the enrichment might be important for the size of the effects (Van de Weerd, 1996), we included a condition with a higher degree of enrichment (superenriched) to act as a positive control. We investigated two different rearing conditions, standard and tissue, to see if early rearing conditions affected immune response or behavior. We also tested mice in an open-field test to obtain additional information about their behavior in a novel environment. The second experiment, in which we tested mice from standard and superenriched conditions, was conducted at a pharmaceutical company, Solvay Pharmaceuticals BV, (Weesp, The Netherlands). We investigated the variability in the results of a physiological routine procedure: stress-induced hyperthermia (SIH), which is normally used to detect possible anxiolytic effects of drugs in mice. Effects of enrichment on handling of the animals also were studied in this experiment. Because housing conditions may have an effect on body weight and food intake (Chvédoff, Clarke, Irisarri, Faccini, & Monro, 1980; Van de Weerd et al., 1997a; Van de Weerd et al., 1999), we monitored these in both studies.

ANIMALS, MATERIAL, AND METHOD

Experiment 1

Animals. The sample comprised 128 male mice (RIVM:N:NIH; RIVM, Bilthoven, The Netherlands). At the start, the animals were approximately 3 weeks of age and weighed 10 to 14 g. Half of these mice ($n = 64$) had been reared under "tissue" conditions (bedding and one tissue, Kleenex©, Kimberly-Clark Corpora-

tion, Veenendaal, The Netherlands), and the other half ($n = 64$) had been reared under standard conditions (only sawdust bedding). After arrival, the mice were housed in groups of 8. One animal from the enriched-housing condition died during the fifth week of the experiment.

Housing conditions. Three different housing conditions were used: tissue, enriched, and superenriched. The tissue cages were wire-topped Makrolon Type II cages (375 cm²; UNO roestvaststaal, Zevenaar, The Netherlands) with a 1-cm layer of sawdust bedding (10/20 BK; BMI, Helmond, The Netherlands) and one Kleenex tissue. The enriched cages were wire-topped Makrolon Type II cages with a 1-cm layer of sawdust bedding, one Kleenex tissue, and a nest box of perforated sheet metal (8 cm × 10 cm × 6 cm) with a metal climbing grid (8 cm × 9 cm) attached to it. The superenriched mice were housed in wire-topped Makrolon Type III cages (840 cm²; UNO roestvaststaal, Zevenaar, The Netherlands) with a 1-cm layer of sawdust bedding, two Kleenex tissues, the nest box with climbing grid, two Aspen wood gnawing blocks (large: 2 cm × 2 cm × 10 cm, small: 1 cm × 1 cm × 5 cm; Tapvei Oy, Kaavi, Finland), a plastic tube (PVC; Ø 7 cm, length: 16 cm), wood-wool (±5 grams Tapvei Oy, Kaavi, Finland), and a stainless steel wire grid floor (16 cm × 11 cm) under the food hopper.

In all groups, food pellets (2122 SRM-A 10µm 0.9M RAD, Hope Farms, Woerden, The Netherlands) and tap water were provided ad libitum. The room temperature was 20 to 24°C, relative humidity was approximately 60%, light intensity varied between 210 lux (at floor level) and 240 lux (at the top cage level). The lights were on from 7:00 a.m. until 7:00 p.m. The ventilation rate was 7 air changes per hour, and a radio played softly in the background.

Experimental design. On the first day of the experiment, the mice were housed (after weaning) in groups of 8 on the basis of their body weight, so that the mean body weight of the groups was equal. Within a rearing condition, the cages were divided randomly over the three housing conditions. Two groups of 8 animals were housed under the tissue conditions, three groups were housed under enriched conditions, and three groups were housed under superenriched conditions. Mice were individually marked. Twice a week, the cages were cleaned and tissues and wood-wool renewed and group food intake was measured by weighing the food. On the second day of the experiment the mice were injected with tetanus toxoid. The total duration of the experiment was 5 weeks. Three days before the end of the experiment, the animals were subjected to an open-field test.

Tetanus vaccine potency test. Part of the quality control of batches of inactivated vaccine produced is the demonstration of potency of these products in an animal model. The potency test of the tetanus toxoid vaccine is based on a serological method. Groups of mice are immunized with serial dilutions of the vac-

cine under study and a reference preparation, respectively. After 5 weeks, animals are bled, and serum samples titrated in the *in vitro* Toxin Binding Inhibition (ToBI) test. In The Netherlands, this test has been accepted officially by the regulatory authorities and has been used routinely since January 1996.

In this experiment only one dilution (12.5 $\mu\text{m}/\text{ml}$) of the tetanus toxoid preparation (0.5 ml of DTA 93/1) was used. At the end of the experimental period, mice were killed by terminal bleeding under halothane anesthesia and serum prepared. The serum samples were analyzed in the Tetanus ToBI test (Hendriksen, Van der Gun, Nagel, & Kreeftenberg, 1988), and this test was used to estimate the immune response.

Open-field test. The open-field test was performed on 2 consecutive days, and the mice were divided into two equal groups. The mice from these two groups were divided evenly and allocated to morning and afternoon groups to rule out possible diurnal effects. The test was carried out in the animal room, and a video system was used to record each test; thus, the experimenter did not have to be present in the testing room. Afterward, the behavior of the mice was scored from the videotape using a behavioral observation software package (Observer®; 1990). The ethogram used (see Table 1) was based on the pilot study (Van de Weerd et al., 1999). The open-field arena consisted of a grey PVC wall (50 cm high) surrounding the circular open field (Ø 90 cm). Two clear Perspex V-shaped objects with holes (Cheese Slice, IMS, Cheshire, England) were placed upside down 25 cm from the center and opposite each other. The illumination level varied between 80 and 170 lux at ground level. An individual mouse was placed in the center of the open field between the two objects. Behavior was recorded for 5 min. Between two tests the apparatus and the objects were cleaned with ethyl alcohol (90%) soaked tissues.

TABLE 1
Experiment 1: Ethogram of the Open-Field Test

<i>Behavior</i>	<i>Definition</i>
Locomotion	Walking with four feet on the floor
Immobility	No body movements for one s or longer, head movements are allowed (includes also freezing: no movement at all)
Interaction with the object	Sniffing or gnawing at the object, rearing against it, sitting or walking under or through it
Climbing	Climbing on the object (with four feet off the ground)
Rearing	Standing on the hind legs or leaning against the wall
Other	Grooming (licking, scratching), sniffing, walking only with the front legs (hind part of the mouse on the same spot)

Experiment 2

Animals. The Experiment 2 sample comprised 50 male mice (BALB/cANnCrI BR, Charles River Wiga, Sulzfeld, Germany). At the start of the experiment they were 3 weeks of age. They were reared without any enrichment. After arrival, they acclimatized in the animal room for 2 days and then were housed in groups of 5. One animal from the enriched-housing conditions died during the fifth week of the experiment.

Housing conditions. Two housing conditions were used: standard and superenriched conditions. Standard housing conditions were wire-topped Makrolon Type III cages (840 cm²; Tecniplast, Rome, Italy) with a 2-cm layer of sawdust bedding (Woody Clean S 8/15, Rettenmaier, Germany). Superenriched cages were Makrolon Type III cages (840 cm²; Tecniplast, Rome, Italy) with sawdust bedding, two Kleenex tissues (Kimberly-Clark Corporation, Veenendaal, The Netherlands), a nest box of perforated sheet metal (8 cm × 10 cm × 6 cm) with a metal climbing grid (8 cm × 9 cm), two Aspen wood gnawing blocks (Tapvei Oy, Kaavi, Finland, large: 2 cm × 2 cm × 10 cm, small: 1 cm × 1 cm × 5 cm), a plastic tube (PVC, Ø 7 cm, length: 16 cm), paper nesting material (Enviro-dri ±10 g; BMI, Helmond, The Netherlands), and a stainless steel wire grid floor (16 cm × 11 cm, mesh size 10 mm² × 10 mm²) under the food hopper. Food pellets (RMH-TM; Hope Farms, Woerden, The Netherlands) and water were provided ad libitum. The room temperature was 20 to 21°C, and the relative humidity was approximately 60%. The lights were on from 7:00 a.m. until 7:00 p.m. Ventilation rate was 16 air changes per hour, and a radio played softly in the background.

Experimental design. On the first day of the experiment, the mice were housed in groups of 5 on the basis of their body weight, so that the mean body weight of the groups was equal. The cages were divided randomly over the two housing conditions. Mice were marked individually with a waterproof text marker on the tail, and this was renewed every week after the mice were weighed. Every week, the cages were cleaned, tissues and paper nesting material were renewed, and group food intake was measured by weighing the remaining food. The total duration of the experiment was 6 weeks; after 5 weeks of housing under the different conditions the mice were tested in the SIH test. For this procedure, the mice had to be housed individually for approximately 24 hr. We measured body weight before and after this isolation to see if mice from different housing conditions reacted differently to isolation.

SIH. Measurements of (rectal) body temperature at repeated intervals cause the body temperature of mice to rise, thus indicating stress. The procedure, described by Van der Heyden, Zethof, and Olivier (1997), can test the effects of dif-

ferent pharmaceutical compounds as well as the reactions of mice to different circumstances, such as housing conditions. Rectal temperatures were measured at 0 min (basal temperature) and 10 min later (the increase in temperature is a stress response to the handling of the mouse). The measurement was carried out by holding a mouse in one hand (45° angle); with the other hand, a thermistor probe was inserted gently for a length of 2 cm into the rectum of a mouse. Digital recordings of the temperature were determined with an accuracy of 0.1°C by means of a Keithley 871A digital thermometer (NiCr–NiAl thermocouple). The probe, dipped into silicon oil before inserting, was held into the rectum until a stable rectal temperature was measured for 20 sec. Temperature in the experimental room was 20°C. Testing was performed between 10:00 a.m. and 12:00 p.m. and 1:00 p.m. and 3:00 p.m. Groups of different housing conditions were evenly allocated to morning or afternoon sessions.

Body weight after isolation. For the SIH procedure, the mice were housed individually in Makrolon Type I cages (216 cm²; UNO Roestvaststaal, Zevenaar) for approximately 24 hr after transportation to the room where the SIH test was going to be performed. They were weighed on a digital balance before and after isolation for the SIH procedure.

Handling. In the last week of the experiment, the mice were videotaped during weighing (a mouse was placed in a small cage on the weighing scale), and tail marking (mouse was held by the tail and text marker was applied) to evaluate their behavior during these procedures. Five phases during these procedures were observed and classified into two or three categories (see Table 2). The score given was an agreement score given by two independent observers who were not familiar with the housing conditions of the mice (not visible on the videotape).

Statistical Analyses for Experiments 1 and 2

We analyzed all collected data for differences in both mean and MAD (variation), using the statistical package SPSS (Version 9.0). Data of individual mice were analyzed; however, “group” was included in the analysis as a factor, taking into account possible variation between the groups. To analyze possible differences in MAD, we subtracted the mean value per group or cage from the individual value of a mouse: $MAD = |X_i - \bar{X}|$. We then carried out statistical analyses, similar to those for the means, on the absolute values of this subtraction.

We subjected individual body weights, body weights before and after isolation, food intake per cage, and SIH to a repeated measures analysis (multivariate Pillai’s test) to detect time effects and possible differences between the housing conditions. We used a multivariate analysis of variance (MANOVA) to analyze possible

TABLE 2
Experiment 2: Behavior Scored During Weighing and Tail Marking Procedures

<i>Phase</i>	<i>Scores</i>
Behavior in weighing cage	1 = Mouse shows few movements and has normal breathing, is explorative 2 = Mouse shows more movements and faster breathing, may show rearing
Behavior during tail marking	
First part	1 = Mouse shows few movements, breathing is normal 2 = Mouse "walks" with forepaws 3 = Mouse "walks" with all four limbs, wants to get away
Second part	Same scores as for first part
Turning of mouse	1 = Mouse does not turn around 2 = Mouse turns around and its position needs to be corrected
Overall impression of mouse	1 = Very relaxed, at ease, few movements, shows normal breathing 2 = Stressed, freezing behavior, fast breathing

effects of the housing conditions on the duration and frequencies of the behavioral elements from the open-field test. To determine which of the four behavioral elements (climbing was omitted because of its low frequency) differed between the housing conditions, we subsequently carried out a Bonferroni-corrected analysis of variance (ANOVA) on each of them. To compare the outcome of the ToBI test, we chose an arbitrary optical density (OD) level of 0.700, just above the OD₅₀ value (the OD₅₀ value is an indirect measure for that dilution of the serum in which half of the added tetanus toxin has been neutralized by the antibodies present in the serum). For each mouse, we calculated the extent to which the serum had to be diluted to achieve an exact optical density of 0.700. We subjected these theoretically calculated values to a MANOVA.

When we found overall differences among the three housing conditions (RIVM experiment), we made multiple comparisons (contrasts) using the Bonferroni correction to determine which differences were significant. Nonparametric scores for the handling procedures were analyzed with a Mann-Whitney *U* test. The overall level of statistical significance was preset at $p < .05$.

RESULTS

Experiment 1

Body weight and variation in body weight. Figure 1 shows mean body weight of the mice in each housing condition and their MADs. Repeated measures

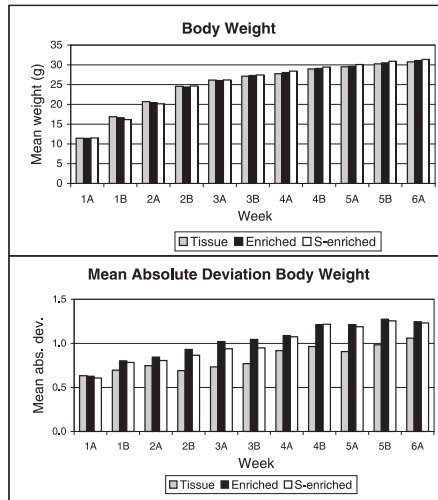


FIGURE 1 Experiment 1: Mean body weight (top) and mean absolute deviations (Mean abs. dev.; bottom) per housing condition. Part A of the weeks consisted of 3 days, Part B of 4 days. $N = 127$. S-enriched = superenriched.

analysis found no overall significant housing effect but did find a significant time effect ($p < .001$). A Time \times Housing interaction ($p < .001$) was found. In the first weeks of the experiment, tissue housed mice gained weight faster; but from Week 3B on, mice from superenriched housing conditions gained weight faster than mice from the tissue and enriched housing conditions. (Part A of the weeks consisted of 3 days; Part B of the weeks consisted of 4 days.) Mice from the enriched housing condition also gained weight faster than the mice from the tissue conditions. A Time \times Housing group interaction ($p < .01$) was found, which means that some groups gained weight faster than others did, but this was not specific for any of the housing conditions.

The MAD (see Figure 1, bottom) between housing conditions did not differ, but again there was a significant time effect ($p < .01$). Repeated measures analysis showed a significant effect for rearing ($p < .05$, data not shown) and a Time \times Rearing interaction ($p < .001$). Mice reared in tissue cages were heavier (from the beginning of the experiment) and gained weight faster than the mice reared in standard cages. No differences were found in the MAD, but there was a significant time effect for rearing ($p < .01$).

Food intake. Repeated measures analysis showed a significant housing effect ($p < .001$) for food intake (data not shown). Comparisons revealed that mice from superenriched housing conditions consumed significantly more food than enriched ($p < .001$) and tissue housed mice ($p < .001$), independent of the rearing con-

ditions. A significant time effect ($p < .01$) was found, indicating that overall food consumption increased with time.

The effect of rearing conditions was significant ($p < .01$); mice reared with tissues consumed more food than mice reared in standard conditions. No MADs could be calculated because food intake was measured per group of mice.

Open-field test duration of behavioral elements and variation in duration. Figure 2 shows the mean duration of the behavioral elements of the open-field test for the three different housing conditions and their mean absolute deviations. The MANOVA showed an overall significant housing effect ($p < .001$). Further analysis (Bonferroni-corrected ANOVAs) revealed that duration of locomotion and interaction with the object were significantly different among the housing conditions (locomotion: $p < .001$, interaction: $p < .001$). Tissue housed mice showed more locomotion than the enriched mice (multiple comparisons, $p < .001$) and superenriched housed mice (multiple comparisons, $p < .001$). Enriched mice also showed significantly more locomotion than superenriched housed mice (multiple comparisons, $p < .01$); however, mice from superenriched conditions showed more interaction with the objects than did the tissue housed mice (multiple comparisons, $p < .001$) and the enriched housed mice (multiple comparisons, $p < .001$). Enriched housed mice showed more interaction than tissue housed mice (multiple comparisons, $p < .001$).

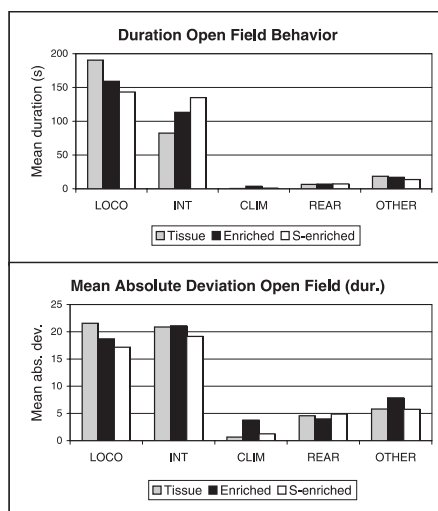


FIGURE 2 Experiment 1: Mean duration of open-field behavior (top) and their mean absolute deviations (Mean abs. dev.; bottom). $N = 127$. LOCO = locomotion; INT = interaction; CLIM = climbing; REAR = rearing; OTHER = other behavior; S-enriched = superenriched.

No significant effects were found for the MADs among the three different housing conditions. Rearing conditions did not show any significant differences in the duration of the behavioral elements or their MADs.

Open-field test frequency of behavioral elements and variation in frequency. Figure 3 shows the mean frequencies of the behavioral elements of the open-field test for the three different housing conditions and their mean absolute deviations.

The MANOVA showed an overall significant housing effect ($p < .001$). Further analysis (Bonferroni-corrected ANOVAs) revealed that frequency of locomotion and interaction with the object were significantly different among the housing conditions (locomotion: $p < .01$, interaction with object: $p < .001$). Mice from the superenriched conditions showed a higher frequency of locomotion (multiple comparisons, $p < .01$) and interaction (multiple comparisons, $p < .001$) than mice from enriched conditions and a higher frequency of locomotion (multiple comparisons, $p < .01$) and interaction (multiple comparisons, $p < .001$) than mice from tissue conditions.

No significant effects were found for the MADs among the three different housing conditions. Rearing conditions did not cause significant differences in the frequency of the behavioral elements or their MADs.

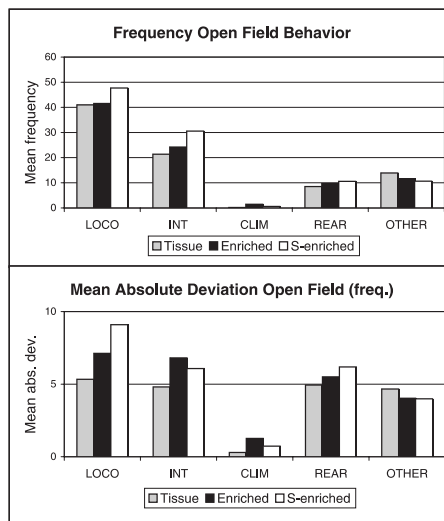


FIGURE 3 Experiment 1: Mean frequency of open-field behavior (top) and mean absolute deviations (Mean abs. dev.; bottom). $N = 127$. LOCO = locomotion; INT = interaction; CLIM = climbing; REAR = rearing; OTHER = other behavior; S-enriched = superenriched.

Immune response and variation in immune response. The univariate ANOVA revealed no significant differences for housing conditions in immune response or their MADs (see Table 3) or for the rearing conditions. A small effect of housing groups ($p < .05$) was found: In some groups, variation was smaller than in other groups, but this was not for a specific housing condition.

Experiment 2

Body weight and variation in body weight. Figure 4 shows the mean body weight of the mice per housing condition and their MADs. Repeated measures analysis found an overall significant housing effect ($p < .001$): Mice from superenriched housing conditions were heavier than those from standard conditions. A significant time effect was also found ($p < .001$). A Time \times Housing interaction ($p < .001$) was found, probably caused by the fact that mice from the superenriched housing conditions gained weight faster than mice from standard housing conditions. The MADs among housing conditions did not differ, but there was a significant time effect ($p < .01$).

Food intake. Repeated measures analysis showed a significant housing ($p < .01$) effect (data not shown). Mice from superenriched housing conditions consumed significantly more food than did standard housed mice. A significant time effect ($p < .001$) was also found, indicating that overall food consumption varied over time.¹

Body weight after isolation and variation in body weight. Figure 5 shows mean body weights of the mice before (Weight 1) and after (Weight 2) being housed individually for approximately 24 hr (isolation) and their MADs. Repeated measures analysis found an overall significant housing effect ($p < .001$): Mice from superenriched housing conditions had overall higher body weight (Weight 1 and Weight 2 together).

A significant time effect also was found ($p < .001$). A Time \times Housing interaction ($p < .05$) was found, indicating that mice from the superenriched housing conditions lost slightly more weight than mice from standard housing conditions. Because mice from superenriched conditions were significantly heavier than mice from standard conditions, they had more weight available to lose. Therefore, we also analyzed relative decrease in body weight and confirmed that mice from superenriched conditions lost more weight than standard housed mice ($p < .05$). The MADs among housing conditions did not differ.

¹No mean absolute deviations could be calculated because food intake was measured per group of mice.

TABLE 3
Experiment 1: Mean Values of Immune Response Test and Their Mean Absolute Deviations for the Different Housing Conditions

<i>Housing Condition</i>	<i>Immune Response</i>	<i>Mean Absolute Deviation</i>
Tissue	.82	.26
Enriched	.77	.28
Superenriched	.91	.38

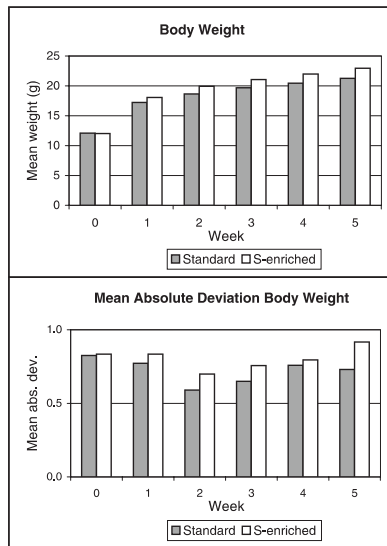


FIGURE 4 Experiment 2: Mean body weight (top) and mean absolute deviations (Mean abs. dev.; bottom) per housing condition. $N = 49$. S-enriched = superenriched.

SIH and variation in SIH. Figure 6 shows mean body temperatures of the mice at Time 0 (SIH0) and 10 min later (SIH10) and their MADs. No overall significant housing effect or Time \times Housing interactions were found with the repeated measures analysis. A significant time effect was found ($p < .001$). The mice reacted to the procedure with a rise in body temperature, which was similar for mice from both housing conditions. The MADs among housing conditions did not differ, but there was a significant time effect ($p < .001$).

Handling. Table 4 shows the results for the handling scores. Mann–Whitney U tests revealed a significant housing effect in the total handling score, which was the sum of all scores together, except for the overall impression score ($p < .01$). The

separate scores that reached significance were the behavior in the weighing cage ($p < .01$) and the overall impression of the state of the mouse ($p < .01$). Behavior during the first phase of tail marking showed a trend ($p = .053$). During all these phases, mice from superenriched housing conditions had lower scores, indicating they were more at ease than mice from standard housing conditions.

DISCUSSION

The enrichment used in this study influenced several parameters. In both experiments, body weight and food intake were affected. The effect on body weight was largest in Experiment 2; mice from the superenriched conditions weighed more than mice from the standard housing conditions. In Experiment 1 the effect was smaller, but mice from superenriched conditions and enriched conditions gained weight faster than mice from tissue housing conditions. Other researchers also have found a significant increase in body weight for enriched housed mice (Chapillon, Manneché, Belzung, & Caston, 1999; Dahlborn, Van Gils, Van de Weerd, Van Dijk, & Baumans, 1996; Manosevitz & Joel, 1973; Van de Weerd,

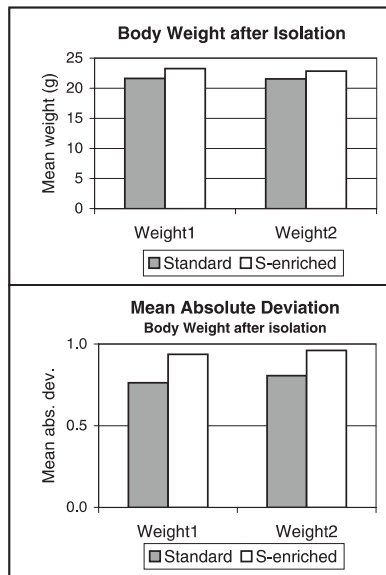


FIGURE 5 Experiment 2: Mean body weight (top) of the mice before (Weight1) and after (Weight2) being housed individually for approximately 22 hr (isolation) and mean absolute deviations (Mean abs. dev.; bottom). $N = 49$. S-enriched = superenriched.

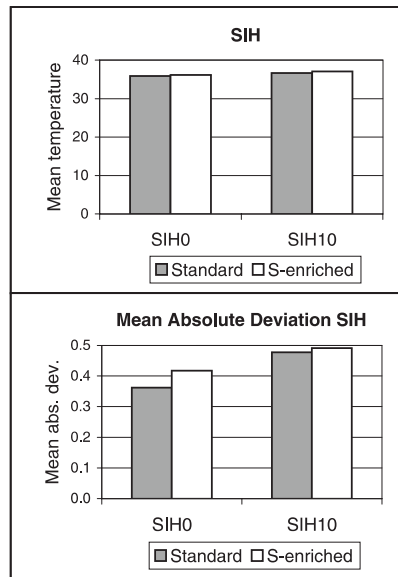


FIGURE 6 Experiment 2: stress-induced hyperthermia (SIH). Mean body temperatures (top) of the mice at Time 0 (SIH0) and 10 min later (SIH10) and mean absolute deviations (Mean abs. dev.; bottom) are depicted. $N = 49$. S-enriched = superenriched.

TABLE 4
Experiment 2: Mean Handling Scores for Different Phases of Handling

Phase	Standard	Superenriched
Behavior in weighing cage	1.71	1.32
Behavior during tail marking, first part	2.08	1.72
Behavior during tail marking, second part	2.62	2.28
Turning of mouse	1.25	1.24
Overall impression of mouse	1.67	1.28

Baumans, Koolhaas, & Van Zutphen, 1994, 1997a). In both experiments, mice from superenriched housing conditions consumed more food, which is consistent with the higher body weights. Higher food intake is not always an effect of enrichment, but the methods and ranges of enrichment vary between different studies. There are no standardized criteria for assessing whether an environment has been enriched (Newberry, 1995). When comparing results from enrichment studies, one must keep in mind that the degree of complexity of the enrichment plays a role in the level of behavioral and physiological effects (Van de Weerd, 1996). Dahlborn et al. showed that mice housed in cages enriched with only

nesting material did not differ from mice housed in standard conditions, in regard to several behavioral and physiological parameters. However, differences were found when mice from environments enriched with both nesting materials and objects were compared with mice from standard environments. The body weight increase reported here might be due to the larger food intake and to the insulation provided by the enrichment. In enriched environments, animals have more options to choose areas of different thermal quality, such as the nesting material and shelters. Thus, there may have been a reduction in maintenance of body heat requirement, and food could be used more efficiently (Van de Weerd et al., 1997a).

An effect of enrichment with tissues also was found in early life; mice reared in an environment with tissues were heavier and consumed more food than mice from the standard (barren) environment. This rearing effect was detectable throughout the whole of the experiment, even though the mice from the two rearing conditions were divided over the three different housing conditions and influences of these environments may have been "added." This might indicate that providing tissues as enrichment in an early stage of life has effects on the metabolism and may have ensured a different regulation of food and energy.

Dahlborn et al. (1996) and Van de Weerd et al. (1999) found that mice from highly enriched cages interacted more with the objects in the open-field test than did standard housed mice. Experiment 1 of this study found that mice from superenriched housing conditions showed more interaction with the objects than the enriched and standard housed mice, whereas mice from the standard conditions and the enriched housed mice showed more locomotion than the superenriched housed mice.

Janus, Koperwas, Janus, and Roder (1995) found higher activity levels in standard housed mice in the more complex of two radial mazes compared with enriched housed mice. They concluded that enriched subjects demonstrated faster habituation to the complex maze. Joseph and Gallagher (1980) presented similar conclusions from rat studies. They concluded that rats reared in restricted environments develop a limited behavioral repertoire, which is dominated by a tendency to overrespond to various stimuli in a testing environment. The higher open-field activity levels of mice from the standard (tissue) conditions in this study and in other studies (Engellenner, Goodlett, Burrig, & Donovick, 1982; Manosevitz & Joel, 1973; Manosevitz & Montemayor, 1972; Van de Weerd, 1996; Van de Weerd et al., 1994) are in concordance with this.

Animals from enriched environments have been found to be less fearful (Chapillon et al., 1999) and more exploratory and seek greater variability or novelty and unfamiliar stimuli than restricted animals (Joseph & Gallagher, 1980). Although the mice from the superenriched environments were already accustomed to objects in their home cage (Riittinen et al., 1986), the objects in the open field were new to them. They interacted significantly more with them and, compared with the

mice from the other housing conditions, showed more frequent locomotion around and between the objects. The interaction with the objects might indicate a higher level of exploration, which agrees with the common assumption in open-field studies that emotionality (or fear) and exploration are inversely related; that is, high emotionality inhibits exploration, and vice versa (Archer, 1973).

The handling scores in Experiment 2 demonstrated that mice from superenriched environments were more "relaxed" and easier to handle than mice from standard housing conditions. This effect was noticed in the first weeks of the experiments. Similar observations were made in Experiment 1; however, handling was not quantified in this experiment. Engellener et al. (1982) also found lower reactivity scores (including resistance to capture, handling, and vocalizations) for mice from enriched conditions compared with mice from restricted (individual) environments. It can be hypothesized that animals from enriched environments will react less fearfully and habituate sooner to handling procedures (Van de Weerd et al., 1997a). Enriched environments may have given them a more secure feeling because they have increased control over events (Wiepkema & Koolhaas, 1993). Rabbits in enriched group housing systems with hiding places were easier to capture (Love, 1994; Whary, Peper, Borkowski, Lawrence, & Ferguson, 1993). However, gerbils from enriched environments with a shelter were more difficult to capture and restrain than gerbils from standard laboratory environments (Clark & Galef, 1980). More research is needed to elucidate the effects of enrichment on handling.

The superenriched housed mice lost more body weight after being housed individually in small cages for a night in Experiment 2 (Van Elderen, 1996). This may indicate that the transition from the enriched environment to the barren and solitary conditions had a greater impact on these animals than on animals from barren conditions, although the variability in the results was not affected. However, in a pilot study, when the mice were monitored over a longer period of time, after the first day the mice from enriched conditions recovered and started gaining weight rapidly. The effect of weight loss was not found in a different strain of mice (Van Elderen, 1996). The reaction of the mice from enriched housing conditions could also just be a physiological reaction to the lack of insulating nesting material and shelter. However, further investigation should reveal whether an animal is more averse to being removed from an enriched environment than to being removed from an environment that lacks these stimuli. Because an enriched environment is beneficial to the animals, it follows that removal of enriching features or transfer to an environment lacking these features may have adverse effects (Newberry, 1995).

Although the movement to individual cages meant a major change of environment for the mice from superenriched environments, we found no difference in reaction to the rectal temperature measurements (SIH). Mice from both housing

conditions reacted to the stress of the procedures with a significant rise in temperature. These results are in concordance with a pilot experiment (Van Elderen, 1996). When the stress is too large or too invasive, as in this SIH procedure, which is a stressful stimulus that activates the hypothalamic–pituitary–adrenal axis and the sympathoadrenal medullary system, there may be limits to the ability to habituate to novel situations by mice from enriched environments (Groenink, Van der Gugten, Zethof, Van der Heyden, & Olivier, 1994).

No effects of the enrichment were found on the immune response, which is in concordance with Van de Weerd et al.'s (1999) pilot study. Kingston and Hoffman-Goetz (1996), however, found effects of enriched environments on the immune system of mice. They suggested that enrichment buffers or dampens the reactivity of the immune system in response to distress and that animals in enriched environments would show less variable, better regulated, or more rapid recovery of immune responses. Differences with this study are that Kingston and Hoffman-Goetz did not induce immunity but studied the basal state of the immune system in enriched environments and the immune response to acute exercise stress.

The environmental enrichment did not influence the variability in any of the parameters measured. A similar result was found in the pilot study at the RIVM (Van de Weerd et al, 1999). When we reevaluated parameters from previous enrichment studies (Van de Weerd et al., 1994, Van de Weerd et al., 1997a) for differences in variability using the method described in this article, it appeared that for some of the parameters of enriched housed mice the variability was lower than for standard housed mice (cage emergence test, blood corticosterone). In some parameters, the variability was larger for enriched housed mice (freezing behavior in the open field, behavior in aluminum foil test); in other parameters, variability did not differ (body weight, adrenal weight, hole board behavior, or other open-field behavioral elements).

Findings in other studies are also similar. Gärtner (1999) found that enrichment enhanced only a proportion of the coefficients of variation he compared for different traits. Eskola et al. (1999) also found increased, decreased, or unchanged variation in a large range of clinical and physiological parameters in a study with Wistar rats. However, their Solo Power analysis seems less suitable for these comparisons because it assumes that the enrichment used does not influence group means (Eskola et al., 1999), which is a risky assumption in studies investigating effects of enrichment. Tsai and Hackbarth (1999) compared variability (by comparing coefficients of variation not using a statistical analysis) of behavioral and physiological parameters of mice from different strains. They concluded that the effect of enrichment on the coefficients of variation (decreased, increased, or unchanged) was different per strain and test. Janus et al. (1995) reported that they had to increase the number of mice from standard con-

ditions to be tested in a four-arm radial maze because these animals showed a greater variability in exploratory behavior; however, this variability in behavior was not found in an eight-arm radial maze. Kuhnen (1998–1999) found no effects of enrichment of hamster cages on the standard deviations of fever-induced body temperature measurements. Zimmerman (1999) analyzed a range of behavioral (open-field and novel object test) parameters and physiological data from rats and found no increase in interindividual variability from enrichment, although she did find a trend that rats from standard individual housing conditions had the highest variability in the behavioral parameters.

All this seems to indicate that the effects of enrichment on the variability in results are parameter dependent. Although some authors stress the fact that more animals might be needed when using enrichment, the results just discussed indicate that in many cases enrichment does not increase variability, might even decrease it, and that a decrease may lead to fewer animals needed. As it appears, no general conclusion about the application of enrichment can be drawn on the basis of the effects on variability. The best course of action when deciding whether to enrich might be to investigate for each study whether enrichment influences the results and, if so, in what way. If the variability is increased, then one should evaluate whether the increase in variability is acceptable when balanced against the enhanced welfare of animals in enriched environments (although this weighing can be difficult). When enrichment does not influence variability or decreases it, there is no reason for not introducing cage enrichment and, thus, contributing to the animals' welfare.

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