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Environmental Modification and Agonistic Behavior in NIH/S Male Mice: Nesting Material Enhances Fighting but Shelters Prevent It

Eila K Kaliste,[1,*] Satu M Mering,[1] and Hannele K Huuskonen[2]

Outbred NIH/S male mice were housed from weaning in groups of 4 without enrichment (control) or with nesting material (nest), nesting material and a box (nest-and-box), or nesting material and a tube (nest-and-tube) as environmental modification. The aim of the study was to investigate effects of widely recommended nesting material and additional shelters on male mice. The aggressiveness of the mice in their home cages clearly increased in the nest group, as assessed by the number of wounds. In the nest group, fighting was a stressful situation for the mice, leading to changes in weight gain and in the weights of the thymus, adrenals, spleen, and epididymal adipose tissue. Moreover, the agonistic behavior of these mice toward an intruder was increased both in individual tests (an intruder with the individual mouse) and group tests (an intruder with a group of mice). The provision of a box or tube as a shelter, in addition to nesting material, prevented intracage fighting and did not lead to alterations in the weight gain or organ weights of the mice. However, the agonistic behavior of mice with shelters was slightly increased in behavioral tests. Anxiety in the elevated plus-maze was not affected by any of the housing systems. In conclusion, the agonistic behavior of NIH/S mice, an aggressive strain, seemed to be easily enhanced by these environmental modifications. The suitability of any enrichment should be carefully evaluated, especially when highly aggressive mice are used.

Abbreviations: GI test, group intruder test; GLM, general linear model; RI test, resident intruder test

Environmental modification of laboratory animals is an important means of improving their quality of life. Accordingly, recent recommendations strongly emphasize the inclusion of enrichment in the cage environment of laboratory rodents.[7] In the literature, there is much experimental support for beneficial effects of enrichment. These include increased play and sociopositive behaviour,[20] reduction of stereotypies,[39] and increased locomotory and exploratory behavior.[26] Quite often, moreover, no differences have been found in the physiologic or behavioral parameters followed in animals housed with or without various modifications.[1,24,34] This lack of effect is regarded as a positive sign: environmental modification does not interfere with animal experiments. In male mice, a typical negative consequence of modification is increased aggressiveness, which has occurred in several studies.[13,20,38] As another negative consequence, environmental modification has been thought to increase variation among laboratory animals and therefore variation in experimental results, leading to the need to increase the number of animals used experimentally. That environmental modification leads to increased variation, decreased variation, and no effect on variation have all been reported.[1,21,31,32,36]

The literature clearly shows how variable the consequences of environmental modifications may be. In male mice, increased aggressiveness may lead to serious problems in their wellbeing, and these problems may also affect their responses in experimental settings. The aim of the present study was to investigate the effects of nesting material and additional modification (a tube or box) on intermale aggression among aggressive, outbred NIH/S mice. Growth, food intake, and organ weights of animals were measured, as well as behavioral variables (agonistic behavior in the home cage, group intruder test, resident–intruder test, and the elevated plus-maze test) in 4 groups of male mice (control, nest, nest-and-box, and nest-and-tube). The effects of housing environment on the within-group variation were evaluated also.

Materials and Methods

Animals and environment. The study was approved by the Animal Care and Use Committee of the University of Kuopio (Finland). All the procedures were performed in agreement with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.[10]

We used 128 (64 resident, 64 intruder) male, outbred NIH/S mice from the SPF barrier colony of the National Public Health Institute (Kuopio, Finland). Animals were housed from weaning in stainless steel, solid-bottom cages ($42 \times 25 \times 15$ cm) with aspen bedding (Tapvei Oy, Kaavi, Finland) in groups of littermates until the beginning of the experiment (at 5 wk of age). Cages were changed once a week. From weaning and during the experiment, mice were housed in a cubicle of a conventional animal room, at an ambient temperature of 21 ± 0.5 °C and relative humidity of 46% ± 1.4%. The light:dark cycle of the animal room was 12:12-h, with lights off at 1200; the light:dark cycle was changed to enable us to perform the behavioral tests during the animals' active time. Pelleted rat and mouse food (R36, Lactamin AB, Stockholm, Sweden) and tap water were available ad libitum. The health status of animals in barrier and conventional facilities were controlled

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[1]National Laboratory Animal Center, University of Kuopio, Kuopio, Finland; [2]National Public Health Institute, Kuopio, Finland.
[*]Corresponding author. Email: Eila.Kaliste@uku.fi

twice a year for the pathogens listed by the Federation of European Laboratory Animal Science Associations,[11] and animals were free from all these pathogens.

Experimental procedures. At the age of 5 wk, animals were allocated randomly into 4 groups, with 16 mice in each group and 4 animals per cage. Three environmental enrichment procedures were used (Figure 1): aspen wood-wool (Tapvei Oy, Kaavi, Finland) as nesting material (about 6 g per cage; nest group); nesting material and a transparent, plastic box (polyvinyl chloride, 11 × 8 × 7 cm; nest-and-box group); and nesting material and a tube (a polycarbonate water bottle with its bottom removed, 7 × 10 cm; nest-and-tube group). Control animals had only bedding material in their cages. The bottles and boxes were washed and new nesting material was provided at each cage change. The experiment lasted for 8 wk, when the animals were 5 to 13 wk of age.

The health and welfare of the animals was checked daily. We assessed the body weight of each animal and the food intake per cage by weekly weighing, that is weight gain (g) per wk and food consumed per cage per wk. The animals were closely inspected once weekly, and the number, size, and severity of wounds in the tail, back, and ventral side were monitored. The wounds found were typically small (1 to 3 mm), well-healed scabs or 1 or 2 larger (4 to 7 mm) areas with healed scabs. However, 2 experimental groups had to be euthanized 2 wk after the experiment started (Figure 2), because of more serious wounding of several cagemates.

During the study, each cage of the experimental groups was video recorded once (S-VHS LC295SN video camera, Grundig, Germany) to obtain data on the general behavior of animals with the enrichment objects. Video recording started when the lights went off at 1200 and was done using a time-lapse recording system at 1-s/min intervals (time-lapse system takes a 1 s recording every min) for 9 h. Three red-painted 25-W lamps were used during the recording. From the videotapes, the number of animals inside, on, or beside the enrichment items, on the cage lid, and elsewhere in the cage (540 observations per cage every 9 h) was monitored using an instantaneous sampling method at 1-min intervals.

Behavioral parameters. ***Elevated plus-maze test.*** A 5-min elevated plus-maze test was conducted for each animal at the age of 12 wk. This test is used widely in pharmacologic research to analyze exploration and anxiety in mice, and it is based on the naturalistic conflict between the tendency of mice to explore a novel environment and the aversive properties of a brightly lit, open area.[15,19,27] The test apparatus had 2 open arms and 2 closed arms (111 × 10 cm). The closed arms had 25-cm high walls, which were covered with black plastic. The plus-maze was elevated 53 cm above the floor. The tests were run between 0830 and 1130 on 2 successive days. Each mouse was placed in the middle of the apparatus with its head facing an open arm, and its behavior was video recorded. The arms of the plus-maze were wiped with mild detergent (Hytox-21, Leverindus, Turku, Finland) after contact with each animal. The total numbers and durations of entries into closed and open arms, latency to the 1st entry into closed and open arms, rearing and grooming behaviors, and the number of stretch-attended postures were analyzed using The Observer software (version 3.0 for Windows, Noldus Information Technology, Wageningen, Netherlands).

Group intruder test. The aggressive behavior of groups of mice was evaluated by using group intruder (GI) tests,[17] modified from the standard opponent method.[5] A 10-min group intruder test was performed with the mice at 12 wk of age. The test was done between 0815 and 0915 by placing 1 unfamiliar naive male mouse of about the same age as the test mice into the home cage of the test animals and video recording the behavior of the animals. Experimental animals were marked with dye on their backs, and the intruder mouse lacked such marking. Nesting material, bottles, and boxes were removed from the cages for the period of testing. From the videotape, the latency, number, and duration of aggressive interactions (biting, fighting, and wrestling) against the intruder and between cagemates were analyzed.

Figure 1. Environmental enrichments used: aspen wood-wool, polyvinyl chloride box, and water bottle without its bottom.

Resident intruder test. A 5-min individual intruder test was performed after the group intruder test, between 0930 and 1215. Each mouse was placed into a small individual mouse cage (30 × 15 × 10 cm, 450 cm^2) immediately after the group intruder test and kept there about 1 h before the resident intruder (RI) test was performed. The RI test is a modification of the standard opponent test[5] and isolation-induced fighting test,[8] in which a standard opponent test is conducted in the home cage of the test mouse. In this experiment, an intruder male mouse (a different opponent than in the GI test) of about the same age as the test mouse, was placed into the cage of the test mouse, and the behavior of the animals was video recorded. Test animals were marked with dye (the same mark as used in the GI test); the intruder mouse lacked such marking. The number, duration, and latency of introductory behavior towards the intruder or resident mice (following, stretched attention, sniffing, or grooming), aggressive behavior against an intruder or resident (aggressive grooming, tail rattling, biting, fighting, or offensive upright postures), and investigation of the environment were analyzed using The Observer software (Noldus Information Technology). The video recordings of 5 test mice were lost due to technical problems.

Euthanasia. Animals were euthanized at 13 wk of age over 3 successive days between 1000 and 1500. After anesthesia of the mice with 70% CO_2: 30% O_2, death was ensured by cervical dislocation. Final body weights as well as the weights of the adrenal glands, spleen, testis, and epididymal adipose tissue were recorded.

Variation in data. The variation in the data is expressed as a coefficient of variance percentage (CV%; [standard deviation/mean] × 100%).

Statistical analysis. Statistical analyses were carried out using the SPSS/PC+ for Windows statistical package (release 6.1.4, SPSS, Chicago, IL). The normality of the data was tested by group

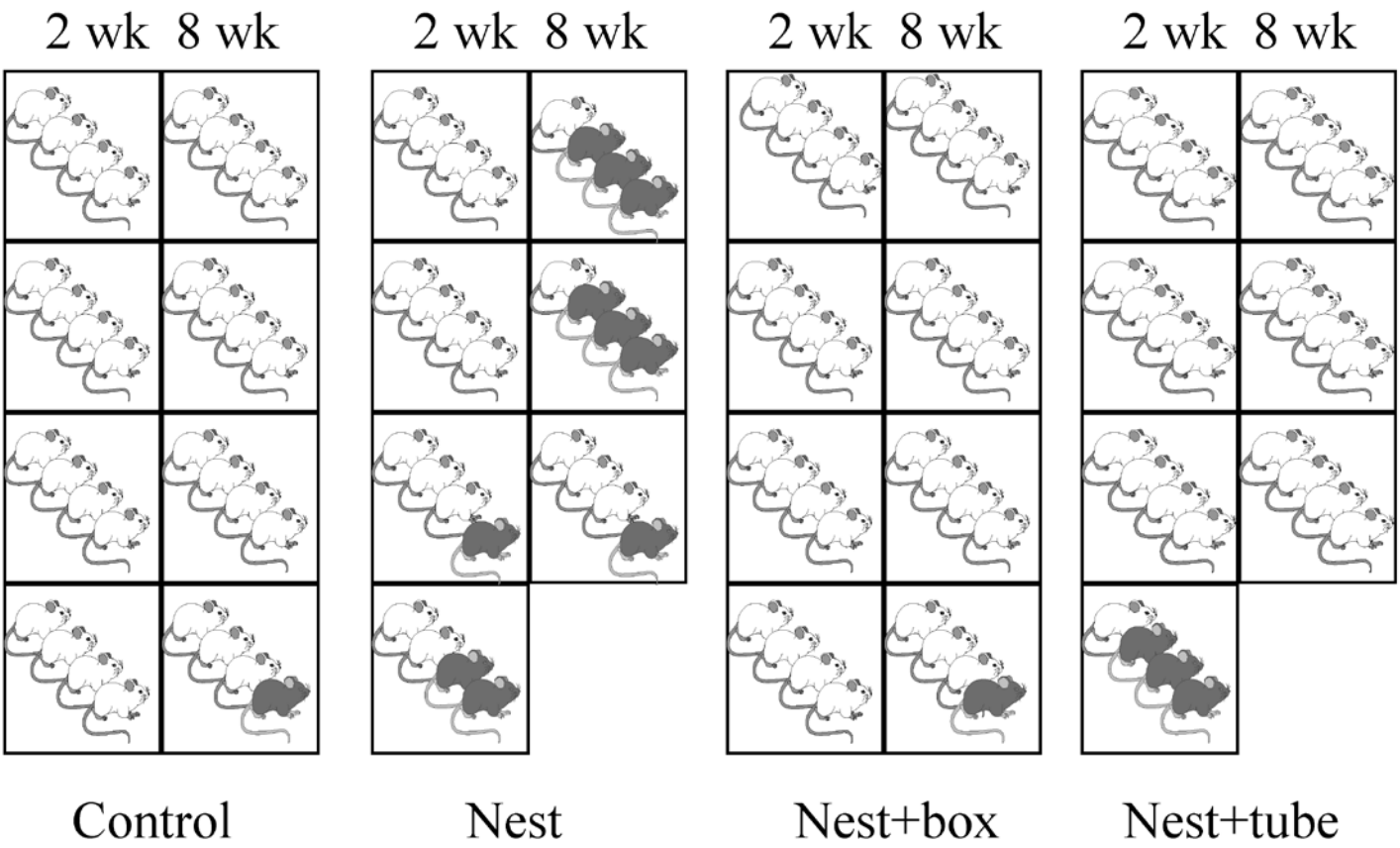

Figure 2. The number of wounded (gray) and nonwounded (white) animals in the 4 groups during the 2nd and 8th wk of the study. The animals from 1 cage each in the nest and nest-and-tube groups had to be euthanized during the 3rd wk of the study because of wounding.

with Kolmogorov–Smirnov tests. The statistical analyses used for normally distributed data were analysis of variance (general linear model [GLM] univariate), multivariate analysis of variance with (GLM repeated measures) or without repeated measures (GLM), and 1-way analysis of variance with Dunnett pair comparison (GLM univariate + Dunnett). Kruskal–Wallis 1-way analysis of variance and multiple comparisons between groups were used when data was not normally distributed. Chi-square and Fisher's exact tests were used for class variables.

Results

Wounds. The occurrence of wounded animals indicated the amount of fighting in groups of mice. In general, aggressiveness between cagemates remained low (Figure 2). The exception was the nest group, in which every cage contained wounded mice. In this group, the animals from 1 cage had to be euthanized at the beginning of the study, because of seriously wounded cagemates. By the end of the study, 7 of the 9 (78%) wounded animals were from the nest group. In addition, 1 cage with excessive fighting was found in the nest-and-tube group, and these animals had to be euthanized 2 wk after the start of the study.

Growth and organ weights. The weight gain of animals was the only parameter with interaction between housing environment and wounds ($P = 0.042$, GLM multivariate; Table 1). Both housing environment and wounding of animals had an effect on weight gain ($P = 0.014$ and 0.03, respectively, GLM univariate). When separately analyzed, a significant difference for housing was found between the control and nest groups ($P = 0.012$, GLM univariate + Dunnett). The animals in the nest group also had enlarged adrenals ($P = 0.009$, GLM multivariate) and reduced epididymal adipose tissue ($P = 0.03$, GLM multivariate), when compared with the animals in the control group.

The data from wounded and unwounded animals is presented in Table 2. Wounds clearly inhibited growth: the weight gain in wounded animals was 53% from the initial body weight and 69% in animals without wounding. Wounding also resulted in increased spleen weight ($P = 0.000$, GLM multivariate) and decreased epididymal adipose tissue weight ($P = 0.010$, GLM multivariate). The adrenals, however, were not enlarged significantly due to wounding ($P = 0.414$, GLM multivariate). Nearly all the wounded animals were in the nest group, in which the weight gain of wounded mice (n = 7) was 10.2 ± 2.8 g and that of unwounded mice (n = 5) was 13.2 ± 2.3 g.

Behavioral parameters. ***General behavior in home cage with enrichment items.*** Animals in the nest group spent less of their time (24% ± 20%) in contact with their enrichment items than did animals in the nest-and-tube (63% ± 10%) and nest-and-box (52% ± 17%) groups (intergroup differences, $P < 0.05$; GLM multivariate; data not shown). The cage lid was used regularly for climbing, with the average time spent in that activity being 7% to 13% of total time (difference between groups not significant, GLM multivariate). When the animals were in contact with the items, they typically were inside the tube (90% of the contact time) or box (85% of the contact time). The rest of the time, they were beside or on top of the items.

Elevated plus-maze test. Neither the environmental enrichment

Table 1. Weight gain and weights of organs (mean ± 1 SD) in mice housed in various environments

	Control (n = 16)	Nest (n = 12)	Nest + box (n = 16)	Nest + tube (n = 12)	General linear model, *P*	
					Housing	Wounds
Initial body weight (g)	19.8 ± 2.9	20.2 ± 2.7	19.7 ± 2.9	19.5 ± 2.7	0.903	0.835
Final body weight (g)	33.9 ± 3.4	31.7 ± 2.2	32.4 ± 2.4	33.9 ± 2.6	0.659	0.415
Weight gain (g)	14.0 ± 2.2	11.4 ± 2.9[c]	12.7 ± 2.7	14.4 ± 1.6	housing × wounds: 0.042[b]	
Adrenals (mg)[a]	2.9 ± 0.88	4.3 ± 0.95[d]	3.0 ± 0.54	3.3 ± 0.49	0.009	0.414
Spleen (mg)[a]	99 ± 37	193 ± 134	101 ± 46	81 ± 13	0.626	0.000
Testis (mg)[a]	185 ± 15	180 ± 15	193 ± 14	187 ± 12	0.172	0.438
Epididymal adipose tissue (mg)[a]	678 ± 319	312 ± 192[e]	526 ± 238	547 ± 150	0.030	0.010

[a]Final body weight taken as a covariate. This covariate had a significant effect on epididymal adipose tissue ($P = 0.000$) and testis ($P = 0.013$).
[b]For housing, $P = 0.014$; for wounds, $P = 0.03$.
[c]$P = 0.012$ (Dunnett test) versus value for control group.
[d]$P = 0.000$ (Dunnett test) versus value for control group.
[e]$P = 0.001$ (Dunnett test versus value for control group.

Table 2. Weight gain and organ data (mean ± 1 SD) for mice with or without wounds

	Wounds (n = 9)	No wounds (n = 47)
Initial body weight (g)	20.4 ± 2.3	19.7 ± 2.8
Final body weight (g)	31.4 ± 2.3	33.3 ± 2.8
Total weight gain (g)	10.9 ± 3.2[a]	13.6 ± 2.6
Adrenals (mg)	4.2 ± 1.2	3.1 ± 0.7
Spleen (mg)	249 ± 124[a]	90 ± 26
Testis (mg)	186 ± 13	187 ± 15
Epididymal adipose tissue (mg)	245 ± 124[a]	582 ± 254

[a]$P < 0.05$ (general linear model; multivariate); see Table 1.

nor the occurrence of wounds had any effect on the behavior of animals in the elevated plus-maze test (data not shown), nor did these 2 parameters interact.

GI test. In the GI test, control animals did not show any aggressiveness toward an intruder or each other, despite the agonistic behavior of the intruders (Table 3). The enrichment items, however, seemed to induce aggressiveness towards a strange intruder: in all 3 groups, there were from 1 to 4 cages that contained individuals that showed aggression toward the intruder. A statistically significant difference in GI test performance occurred only between the nest-and-box and control groups ($P = 0.014$, Fisher's exact test), where fighting occurred in all 4 cages. Fighting between cagemates occurred occasionally in all groups with modifications but not in control group (difference not significant).

RI test. In individual RI tests, control animals showed only low aggression toward intruders: only 2 mice attacked their intruder (Table 4). In contrast, mice with housing modification showed variable aggression in this test (5 to 9 animals/group; intergroup difference [chi square test], $P = 0.045$). However, the aggressiveness of mice from modified housing was also quite low in this test. Only the animals in the nest group showed pronounced aggressiveness toward intruders, with shorter latency time and increased number of attacks ($P = 0.044$, Kruskal–Wallis analysis of variance, but post hoc testing did not find significant differences between the groups). No differences were found between the housing environments in the amount of social contact or defensive behavior in this test, nor had wounds any influence on aggressiveness or the other behaviors measured.

Table 3. Results of group intruder tests

	Residents against intruder	Residents between cage mates	Intruder against residents
Control (4 cages)			
Cages/animals	0/0	0/0	2/8
No. of attacks (duration)	0 (0)	0 (0)	23 (35)
Nest (3 cages)			
Cages/animals	2/3	2 / 3	2/2
No. of attacks (duration)	19 (36)	18 (22)	3 (4)
Nest + box (4 cages)			
Cages/animals	4[a]/6	1/2	4/12
No. of attacks (duration)	16 (29)	4 (3)	27 (67)
Nest + tube (3 cages)			
Cages/animals	1/2	1/3	0/0
No. of attacks (duration)	20 (27)	23 (32)	0 (0)

An intruder mouse was placed in the home cage of resident mice for 10 min. The total number of cages/number of animals in each group with attacks and the total number of attacks (and duration [in s]) are shown.
[a]$P = 0.014$ (Fisher's exact test) compared with value for control group

Variation in data. The variation in the measured parameters expressed within experimental cages is shown as coefficient of variance percentages (CV%) in Table 5. Depending on the parameter, the CV% of body and organ weights was typically 10% to 50%. CV%'s of final body weight, weight gain, and adrenal weight were not affected by the housing environments or wounding. Epididymal adipose tissue and spleen weights were, however, sensitive to the changes in housing environment (the effect of group $P = 0.02$ and $P = 0.06$, GLM multivariate analyses, respectively). The greatest variation in both parameters was in the nest group (epididymal adipose tissue, 53% ± 16%; spleen, 59% ± 6%) but was statistically significant in post hoc testing only for the spleen CV% (Dunnett T3, $P < 0.05$) in comparison with the nest-and-tube group (spleen, 15% ± 6%). Wounding had significant effect on the CV% of spleen ($P = 0.001$, GLM multivariate analysis). From the parameters measured in the elevated plus-maze test, the time spent in open arms showed the greatest variation. However, there was no statistical difference in CV% that was due to housing modifications or wounding. CV%'s for parameters of the GI or RI

Table 4. Results of 5-min resident intruder tests at the age of 12 weeks

	Control (n = 12)	Nest (n = 11)	Nest + box (n = 12)	Nest + tube (n = 16)	Housing[a]	Wounds[a]
No. of aggressive mice	2	8	9	5	0.045	0.578
Aggression						
Latency (s)	253 ± 86	132 ± 114[b]	218 ± 89	212 ± 113	0.044	0.187
No. of events	1 ± 2	9 ± 8[b]	5 ± 7	3 ± 6	0.044	0.160
Time (%)	3 ± 8	15 ± 16	11 ± 16	8 ± 16	0.081	0.133
Social contact						
No. of events	10 ± 4	12 ± 3	9 ± 3	10 ± 4	0.206	0.548
Time (%)	41 ± 18	37 ± 11	41 ± 21	29 ± 10	0.294	0.517
Defense						
No. of events	4 ± 6	1 ± 3	1 ± 1	1 ±1	0.915	0.170
Time (%)	18 ± 38	5 ± 10	2 ± 4	3 ± 6	0.940	0.157

Data are shown as mean ± 1 SD.
[a]Values presented are the results of Kruskall–Wallis analysis of variance, with the exception of those for number of aggressive mice, which arose from chi-square analysis.
[b]$P = 0.056$ (Dunnett T3) relative to control value.
[c]$P = 0.064$ (Dunnett T3) relative to control value.

tests were not calculated because of the nonparametric nature of the data.

Discussion

Research in the field of environmental enrichment has focused on effects on animal physiology and behavior due to environmental modifications. The recent review by Olsson and Dahlborn[24] reviewed 40 studies carried out from 1987 to 2000 and showed the great variability in experimental settings and parameters monitored in the research on enrichment and laboratory mice. The main conclusions of the authors were that mice prefer a more complex cage to the standard cage and that mice should have access to nesting material (examples of nesting materials in reference 33). No negative consequences on behavior or physiology had been found to result from the provision of nesting material for mice. In our present study with NIH/S mice, however, nesting material in the absence of other modifications clearly enhanced fighting in groups of mice (Figure 2).

Increased aggressiveness has occurred frequently in male mice when environmental modifications with complex cage structures have been studied.[13,20,37] The reactions of animals to cage modifications are known to depend on strain.[6,23,35] The outbred NIH/S strain, originating from the Swiss mouse, is very aggressive.[14] Aggression among mice of this strain may be sensitive to environmental modifications. In our previous study with inbred BALB/c and C57BL/6J mice, provision of the same nesting material in similar housing systems did not affect intracage aggressiveness, as monitored by the number of wounds.[9]

Strain differences in reactions to enrichment have recently also been demonstrated by Marashi and colleagues:[20] enrichment induced aggressive behavior in the home cage of CS mice, together with elevated levels of stress hormones (corticosterone and tyrosine hydroxylase). In docile ABG mice, however, similar housing with enrichment did not affect these parameters.[21] With both of these strains, increased play behavior and activity were found as positive effects. The authors conclude that environmental enrichment, and nesting material, is recommended for most mouse strains. With mouse strains that are known to be aggressive, however, the suitability of enrichment should be carefully evaluated and different solutions should be considered.

In our study, provision of a tube or box in addition to the nesting material prevented fighting. Both of these items presumably served as shelters for the mice. Van de Weerd and coworkers[36] concluded that the design of a shelter should be appropriate for each species in order to minimize aggression. In our study, the tube (an old water bottle from which the bottom was removed) seemed to be used frequently by the mice. It prevented the increased aggression induced by the nesting material, and the variations in the data measured were, in most cases, the lowest found. Overall, the box seemed to be equally as effective as the tube in preventing negative effects on organ weights and fighting. However, in GI test the mice in the nest-and-box group showed increased aggressiveness.

In regard to animal maintenance, tube use is practical, economical, and environmentally friendly, because it recycles readily available broken water bottles, which otherwise would be thrown away. The bottom of each bottle was removed to allow animals to run through it. No one mouse can 'own' the bottle and guard it from cagemates. The box we used was not as practical, because it was made of softer plastic and was not as durable as water bottles. However, durable nest boxes, which may be as practical as the water bottle, are commercially available.

The sensitivity of NIH/S mice to responding aggressively to environmental changes was seen also in their reactions during behavioral tests in which individual aggressiveness was measured: living in groups with fighting (that is, the nest group) increased the aggressiveness of the animals in the RI test (Table 4). In addition, the other environmental modifications seemed to increase aggressiveness in the behavioral tests: animals from enriched groups displayed aggressive reactions in GI tests, in contrast to control animals. In the individual RI test, the number of animals with aggressive behavior was greater in enriched groups than in the control groups. The presence of resources (nesting material and shelters) appears to have made the mice more territorial.

Table 5. Coefficient of variance percentage (%) for various parameters

	Control (n = 4 cages)	Nest (n = 3 cages)	Nest + box (n = 4 cages)	Nest + tube (n = 3 cages)	*P*[a]	
					Housing	Wounds
Final body weight (g)	7 ± 5.3	7 ± 0.6	7 ± 3.8	7 ± 0.3	0.711	0.477
Weight gain (g)	17 ± 2.3	27 ± 1.8	16 ± 13.0	12 ± 3.3	0.169	0.434
Adrenals (mg)	27 ± 9.6	21 ± 10.4	27 ± 34.3	16 ± 0.4	0.303	0.862
Spleen (mg)	24 ± 23.6	59 ± 5.9[b]	32 ± 21.0	15 ± 6.0[b]	0.060	0.001
Epididymal adipose tissue (mg)	25 ± 11.9	53 ± 16.2	42 ± 3.3	27 ± 8.4	0.020	0.107
Elevated plus maze						
Time in open (%)	70 ± 24.0	37 ± 1.6	55 ± 10.4	53 ± 22.2	0.127	0.134
Total no. of entries	27 ± 3.8	28 ± 8.5	32 ± 8.0	26 ± 26.1	0.816	0.727
No. of stretch-attended postures	20 ± 7.2	21 ± 10.6	14 ± 2.8	26 ± 15.1	0.347	0.715

Data presented as mean ± 1 SD.
[a]All values presented for wounds are the results of multivariate general linear model tests, as are those for housing, with the exception of those for spleen and epidiymal adipose tissue, which are the results of Kruskal–Wallis tests.
[b]$P < 0.05$ (Dunnett T3) between groups.

Therefore environmental modification may affect the reactions of animals in experimental settings, with potential effects on experimental results.

Aggressive interactions in a mouse group can be assumed to cause stress for the group members. Indeed, social stress induced by social defeat experiences or social hierarchy is widely used as a model for psychosocial stress.[3,30] In the social hierarchy model, establishing and maintaining dominance in a group setting is enhanced by experimental settings, and it is thought to be psychologically and physically stressful for all parties, including both the dominant and subordinate animals. From this research, several consequences of social stress on both the behavior and physiology of rodents have been reported. These include reduced weight gain; decreased epididymal fat or adipose tissue, thymus, and testes weights; and increases in the weights of the adrenals and spleen.[3,30] In our study, living with nesting material as enrichment resulted in reduced weight gain and epididymal adipose tissue weight and in larger adrenals than in control animals (Table 1), all of which are indicators of stress. Moreover, wounding clearly affected these parameters of animals: wounded animals had decreased weight gain, reduced epididymal adipose tissue weight, and enlarged spleens as a result (Table 2). The other housing modifications, however, did not induce stress-related changes. Therefore, the stress induced by nesting material was reduced by the provision of a box or tube as shelter in the cage environment. In related work, Marashi and colleagues[20] reported that enrichment increased the number of agonistic interactions and serum levels of corticosterone and adrenal tyrosine hydroxylase in male mice. However, the stressfulness of the enriched housing was unclear, because enriched housing conditions also were associated with increased play behavior and general activity, which were regarded as positive signs of welfare and might explain the elevated hormonal levels. Moreover, neither the size of enriched cages nor degree of enrichment affected the agonistic behavior. More recently, it has been stated that male mice without general aggressiveness can be housed using half of the space recommended in the ILAR *Guide*[16] without harmful effects.[29] Moreover, the aggressiveness of the FVB male mice in that study (which did fight) was not dependent on their housing density or the floor space available.[29]

Social stress has been reported to increase anxiety.[2,4] Moreover, aggression and anxiety in male mice are thought to be associated: highly aggressive strains (Wild, Swiss-CD1) had lower levels of anxiety than did less aggressive (DBA/2 and C57BL/6N) strains.[25] Highly aggressive dominant male mice displayed higher levels of anxiety in the elevated plus-maze than did subordinate mice.[12] In mice of the CBA/Lac strain, which display low aggression and high emotionality, repeated positive fighting experiences led to increased plus-maze anxiety and aggressive behavioral reactivity toward conspecifics.[18] In our study, however, anxiety in the elevated plus-maze was similar in all test groups, despite the increased aggressiveness of mice in the nest group (Table 3). Moreover, wounding did not affect behavior in the plus maze. Therefore, the sensitivity to induced anxiety in NIH/S mice was not as great as the sensitivity to increased aggressiveness.

Improvement of the housing environment by enrichment might affect the anxiety or emotionality of animals. When housed in enriched environments, anxiety during the elevated plus-maze test and free exploration were reduced in BALB/C and C57BL/6 mice, as indicated by shortened latency and more time spent in open arms.[6] In a 2nd study by the same research group, however, these effects were not confirmed: the BALB/C mice were reported to be more active in the elevated plus-maze, but their general anxiety was not affected.[28] Similarly, another study[1] using a light–dark test with BALB/c and C57BL/6J found no effect on anxiety. As with aggressiveness, the sensitivity to changes in anxiety in different environments seems to be strain- and context-dependent.

Finally, the variability of experimental results is an important concern in scientific research. Modifications of the housing environment have frequently been reported to increase the variation in various experimental results, although no effect or the opposite effect have also been found.[1,22,31,32] We found large differences in coefficients of variation, especially regarding the weights of epididymal adipose tissue and spleen. These were the variables most affected by housing conditions; therefore, they in general seem to be sensitive to environmental (or other) factors. Here again, the sensitivity to changes may vary among strains and depend on the measurement used.[1,31] In data from elevated plus-maze tests, the CV%'s were not affected remarkably by the different housing environments, indicating the low sensitivity of NIH/S mice to changes in anxiety.

References

1. **Augustsson H, van de Weerd HA, Kruitwagen CL, Baumans V.** 2003. Effect of enrichment on variation and results in the light/dark test. Lab Anim **37:**328–340.
2. **Avgustinovich DF, Gorbach OV, Kudryavtseva NN.** 1997. Comparative analysis of anxiety-like behavior in partition and plus-maze tests after agonistic interactions in mice. Physiol Behav **61:**37–43.
3. **Bartolomucci A, Palanza P, Sacerdote P, Panerai AE, Sgoifo A, Dantzer R, Parmigiani S.** 2005. Social factors and individual vulnerability to chronic stress exposure. Neurosci Biobehav Rev **29:**67–81.
4. **Beitia G, Garmendia L, Azpiroz A, Vegas O, Brain PF, Arregi A.** 2005. Time-dependent behavioral, neurochemical, and immune consequences of repeated experiences of social defeat stress in male mice and the ameliorative effects of fluoxetine. Brain Behav Immun **19:**530–539.
5. **Brain P, Poole A.** 1974. Some studies on the use of 'standard opponents' in intermale aggression testing in TT albino mice. Behavior **50:**100–110.
6. **Chapillon P, Manneche C, Belzung C, Caston J.** 1999. Rearing environmental enrichment in two inbred strains of mice: 1. Effects on emotional reactivity. Behav Genet **29:**41–46.
7. **Council of Europe.** 2004. Draft Appendix A of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123). GT 123 (2004) 1 rev 3, Strasbourg, 3 May.
8. **Crawley JN, Schleidt WM, Contrera JF.** 1975. Does social environment decrease propensity to fight in male mice? Behav Biol **15:**73–83.
9. **Eskola S, Kaliste-Korhonen E.** 1999. Aspen wood-wool is preferred as a resting place but does not affect intracage fighting of male BALB/c and C57BL/6J mice. Lab Anim **33:**108–121.
10. **ETS 123.** European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg, 18.III.1986, adopted in Finland 31.V.1990.
11. **FELASA Working Group on Health Monitoring of Rodent and Rabbit Colonies.** 2002. Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. Lab Anim **36:**20–42.
12. **Ferrari PF, Palanza P, Parmigiani S, Rodgers RJ.** 1998. Interindividual variability in Swiss male mice: relationship between social factors, aggression, and anxiety. Physiol Behav **64:**821–827.
13. **Haemisch A, Voss T, Gärtner K.** 1994. Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male DBA/2J. Physiol Behav **56:**1041–1048.
14. **Hilakivi-Clark LA, Lister RG.** 1992. Are there preexisting behavioural characteristics that predict the dominant status of male NIH Swiss mice (*Mus musculus*)? J Comp Psychol **106:**184–189.
15. **Hoggs S.** 1996. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav **54:**21–30.
16. **Institute for Laboratory Animal Resources.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academy Press.
17. **Kaliste-Korhonen E, Eskola S.** 2000. Fighting in NIH/S male mice: consequences for behaviour in resident–intruder tests and physiological parameters. Lab Anim **34:**189–198.
18. **Kudryavtseva NN, Bondar NP, Avgustinovich DF.** 2002. Association between experience of aggression and anxiety in male mice. Behav Brain Res **133:**83–93.
19. **Lister RG.** 1987. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology **92:**180–185.
20. **Marashi V, Barnekow A, Ossendorf E, Sachser N.** 2003. Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. Horm Behav **43:**281–292.
21. **Marashi V, Barnekow A, Sachser N.** 2004. Effects of environmental enrichment on males of a docile inbred strain of mice. Physiol Behav **82:**765–776.
22. **Mering S, Kaliste-Korhonen E, Nevalainen T.** 2001. Estimates of appropriate number of rats: interaction with housing environment. Lab Anim **35:**80–90.
23. **Nevison CM, Hurst JL, Barnard CJ.** 1999. Strain-specific effects of cage enrichment in male laboratory mice (*Mus musculus*). Anim Welf **8:**361–379.
24. **Olsson AS, Dahlborn K.** 2002. Improving housing conditions for laboratory mice: a review of 'environmental enrichment'. Lab Anim **36:**243–270.
25. **Parmigiani S, Palanza P, Rodgers J, Ferrari PF.** 1999. Selection, evolution of behavior and animals models in behavioral neuroscience. Neurosci Biobehav Rev **23:**957–970.
26. **Prior H, Sachser N.** 1994/95. Effects of enriched housing environment on the behaviour of young male and female mice in four exploratory tasks. J Exp Anim Sci **37:**57–68.
27. **Rodgers RJ, Dalvi A.** 1997. Anxiety, defense, and the elevated Plus-maze. Neurosci Biobehav Rev **21:**801–810.
28. **Roy V, Belzung C, Delarue C, Chapillon P.** 2001. Environmental enrichment in BALB/c mice: effects in classical tests of anxiety and exposure to a predatory odor. Physiol Behav **74:**313–320.
29. **Smith AL, Mabus SL, Muir C, Woo Y.** 2005. Effects of housing density and cage floor space on three strains of young adult inbred mice. Comp Med **55:**368–376.
30. **Tamashiro KLK, Nguyen MMN, Sakai RR.** 2005. Social stress. From rodents to primates. Front Neuroendocrinol **26:**27–40.
31. **Tsai PP, Pachowsky U, Stelzer HD, Hackbarth H.** 2002. Impact of environmental enrichment in mice. 1: Effect of housing conditions on body weight, organ weights and haematology in different strains. Lab Anim **36:**411–419.
32. **Tsai PP, Stelzer HD, Hedrich HJ, Hackbarth H.** 2003. Are the effects of different enrichment designs on the physiology and behaviour of DBA/2 mice consistent? Lab Anim **37:**314–327.
33. **Van de Weerd HA, Van Loo PL, van Zutphen LFM, Koolhaas JM, Baumans V.** 1997. Preferences for nesting material as environmental enrichment for laboratory mice. Lab Anim **31:**133–143.
34. **Van de Weerd HA, Van Loo PL, van Zutphen LFM, Koolhaas JM, Baumans V.** 1997. Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. Physiol Behav **62:**1019–1028.
35. **Van de Weerd HA, Baumans V, Koolhaas JM, Van Zutphen LFM.** 1994. Strain specific behavioural response to environmental enrichment in the mouse. J Exp Anim Sci **36:**117–127.
36. **Van de Weerd HA, Van Loo PL, Baumans V.** 2004. Environmental enrichment: room for reduction? Altern Lab Anim **32:**69–71.
37. **Van Loo PLP, Mol JA, Koolhaas JM, Van Zutphen BFM, Baumans V.** 2001. Modulation of aggression in male mice: influence of group size and cage size. Physiol Behav **72:**675–683.
38. **Van Loo PLP, Kruitwagen CLJJ, Koolhaas JM, van de Weerd HA, Van Zutphen LFM, Baumans V.** 2002. Influence of cage enrichment on aggressive behaviour and physiological parameters in male mice. Appl Anim Behav Sci **76:**65–81.
39. **Würbel H, Freire R, Nicol CJ.** 1998. Prevention of stereotypic wire-gnawing in laboratory mice: effects on behaviour and implications for stereotypy as a coping response. Behav Process **41:**61–72.