PREFERENCES FOR NEST BOXES AS ENVIRONMENTAL ENRICHMENT FOR LABORATORY MICE

HA Van de Weerd[1], PLP Van Loo[1], LFM Van Zutphen[1], JM Koolhaas[2] and V Baumans[1]

[1]Department of Laboratory Animal Science, Utrecht University
[2]Department of Animal Physiology, University of Groningen

Animal Welfare 1998, 7: 11-25

SUMMARY

In nature mice live in burrows with nest chambers, where they breed and may hide for predators. In the laboratory a shelter or refuge is an easily applicable form of enrichment, which may enhance the welfare of laboratory mice by giving them more control over their environment. A nest box provides an opportunity to withdraw actively from frightening stimuli outside or inside the cage.

Six nest boxes made of different materials were evaluated in a preference test with male and female mice of two strains (C57BL/6J and BALB/c). In general mice showed a preference for cages with a nest box made of grid metal as compared to clear or white perspex nest boxes, or no nest box and a preference for a cage with a nest box of perforated metal as compared to nest boxes made of grey PVC or sheet metal, or no nest box. When testing a nest box with one open side against a nest box with two open sides, most mice preferred the nest box with one open side and were observed to lie with their heads directed towards the opening.

The results of this study show that nest boxes may be used for enrichment purposes, although it is not entirely clear yet what the main determining features for the animal's choice are. When providing nest boxes as shelters, the structure and design of this type of enrichment should be taken into account, because these may have an effect on the social structure of groups of mice.

INTRODUCTION

Habitat selection or nest-site choice by mice is an important form of behaviour oriented towards the individual's maintenance and survival and its reproductive success (the nest is the most suitable place for breeding), and as a consequence, this choice behaviour is vital to the survival of the species (Buhot-Averseng 1981). In nature, wild rodents live in complex burrows, consisting of tunnels and nest chambers in which they establish their nests (Adams & Boice 1981). When nest boxes were placed in the ground in woods, within 24h, at least 75% of them were visited by rodents, using the nest boxes for different activities: as a feeding post, for storage of food, for the construction of a nest or for bearing and raising offspring (Ryszkowski & Truszkowski 1970; Truszkowski 1974). When mice are startled they will flee to the nearest cover or to their nest if it is not too far away (Schleidt 1951). Laboratory mice explored a novel arena more when a burrow-like shelter was present and they used the burrow for hiding when a predator model was introduced (Birke et al 1985).

Most environments of laboratory animals have been designed to serve human convenience, with little consideration for the animal’s nature (Van de Weerd et al 1994). Environmental enrichment (i.e. additions to an animal’s environment with which it can interact), provides a more structured environment which enables laboratory animals to express more of their species-specific behavioural patterns and thus may enhance their well-being (Beaver 1989; Scharmann 1991). Providing a shelter or refuge gives laboratory animals more control over their environment because it gives them the opportunity to actively withdraw from frightening stimuli outside or inside their cage (e.g. aggressive conspecifics), as well as hide from too much light (Van de Weerd & Baumans 1995). Different types of shelters have been provided in the home cages of laboratory mice for enrichment purposes, eg old drinking bottles (Ward & DeMille 1991), plastic tubes (Peters & Festing 1990), a perspex nest box (Van Loo et al 1996) or a PVC labyrinth (Haemisch et al 1994). Most of those shelters, however, are not very practical in the daily care of laboratory animals. Moreover, there is very little systematic research on the type of shelter required. The utilisation by the animal of a provided object can be measured with the use of behavioural observations or automatic measurements of behaviour (Büttner 1993).

Measuring the preferences of animals is a method of assessing what the animal regards as being better for its welfare and it leads to possibilities for designing better housing conditions (Broom 1988). In this context choice tests have been used in various ways, Blom et al (1992, 1995) studied the relative preference or avoidance for bedding and other housing conditions in mice and rats, and Ottoni & Ades (1991) studied preferences for nest boxes in relation to food and nesting material in hamsters. In an earlier study, Van de Weerd et al (accepted/a) assessed the preference of mice for nesting material to be used as enrichment. Buhot (1981, 1986, 1987, 1989) studied choice behaviour of mice in order to get information about the perceptual abilities of mice to use the spatial properties of their environment for nest-establishment. In a number of studies individual mice or mice in groups were offered nest boxes differing in size, shape and material.

In the present study the preference of laboratory mice for different types of nest boxes was studied in order to test which type of nest box could be used as enrichment for laboratory mice. Mice were offered a free choice for either a cage with or without a nest box, by offering four cages similar in dimensions and contents (food, water and bedding), except for the presence of a nest box.

The experiments of Buhot (1981, 1986) using nest boxes with different shapes revealed a strong preference of mice for nest boxes with a rectangular shape. Adams & Boice (1981) allowed mice to dig burrows in burrow boxes, where it appeared that laboratory mice made almost identical complex burrows as wild mice. Individual mice of the two inbred strains used, made nest chambers which all had the typical dimensions of 8x10x6 cm. Therefore, in the present study rectangular nest boxes with 8x10x6 cm dimensions, but made of different materials, were tested as potential sources of enrichment for laboratory mice in a preference test. The preference for open (two open sides) or closed (one open side) nest boxes was also studied, as natural burrows of mice contain more than one opening (Adams & Boice 1981; Dudek et al 1983).

ANIMALS AND METHODS

Animals

Female and male mice of two strains (C57BL/6JIcoU and BALB/c AnCrRyCpbRivU, N=47) were used. The animals had previously been used in tests studying preferences for nesting material, the time span between those tests and the ones described here was a period of 18 weeks. At the start of the experiment the mice were 26-28 weeks of age. The experiment was conducted in two cohorts, the first experiment (female mice, n=24) lasted seven weeks, the second (male mice, n=23) lasted eight weeks. One male BALB/c mice died before the experiments started.

The animals were housed (per strain and sex) in groups of six animals (and one group of five) in a housing system consisting of two Macrolon type II cages (375 mm^2, UNO Roestvaststaal, Zevenaar, The Netherlands), connected with a passage tube, similar to the tubes used in the preference test system. Both cages were supplied with food-pellets *ad lib* (RMH-B, Hope Farms, Woerden, The Netherlands), tap water *ad lib* and sawdust bedding (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle). The animals were kept in conventional rooms with controlled photoperiod (12:12 L:D, lights on at 07.00 h, approx. 200 lux at 1 m above the floor), temperature (20-22 ^{o}C), relative humidity (50-60 %) and ventilation (15 air changes h^{-1}). Environmental conditions in the experimental rooms were similar, except for the light intensity which was approximately 300 lux at 1 m above the floor, in order to approach light intensities in standard animal rooms.

Preference test system

The preference test system used in this study has been validated and described in detail by Blom et al (1992). In short, a multiple housing system was used consisting of either two or four test cages (Macrolon type II) connected by non-transparent tubes (PVC, inner dimensions: 2.6x2.6x25 cm) to a central cage (15x15x18 cm, transparent perspex). When testing with a two-cage system the central cage was divided diagonally by a PVC sheet (19x17 cm). A total of six multiple housing systems were used divided over two four-tiered constructions in two similar experimental rooms. Each construction was turned gently during testing to prevent bias due to external influences in the experimental room which could interfere with the choice behaviour of the mouse.

The test cages were supplied with 50 g of sawdust bedding (Lignocel 3/4), a food hopper with equal amounts of food pellets (100 g, RMH-B) and tap water in bottles. The central cage had no food, water or bedding. The movements of the mice between the test cages were detected automatically by means of photo-electrical devices in the passage tubes and sent to a computer which calculated the dwelling times per cage (software: Gate-Watch, Metris System Engineering, Wassenaar, The Netherlands).

Behavioural observations

One of the six multiple housing systems was equipped with a video camera system. Each test cage, including the central one, was provided with a video camera (Panasonic WV-1510). The cameras were connected with the photo-electrical devices, so the movements of the mouse could be followed in the test system. The signals from the video cameras were sent to a time-lapse video recorder (Panasonic AG-6700) which could record 24 h of testing (recording: 1/9 of normal speed). During the night the experimental room with the video equipment was illuminated with red light (approx. 5 lux at 1 m) to enable video recordings.

Procedure and nest boxes

Mice were introduced into the test system between 15.00 and 17.00 h and tested individually during 48 h. A group of six mice (of one sex and one strain) was tested simultaneously. Food and water of each test cage were weighed before and after the experiment.

Three test series were performed to test six nest boxes made of different materials, with the following dimensions: 8x10x6 cm (see Figure 1).

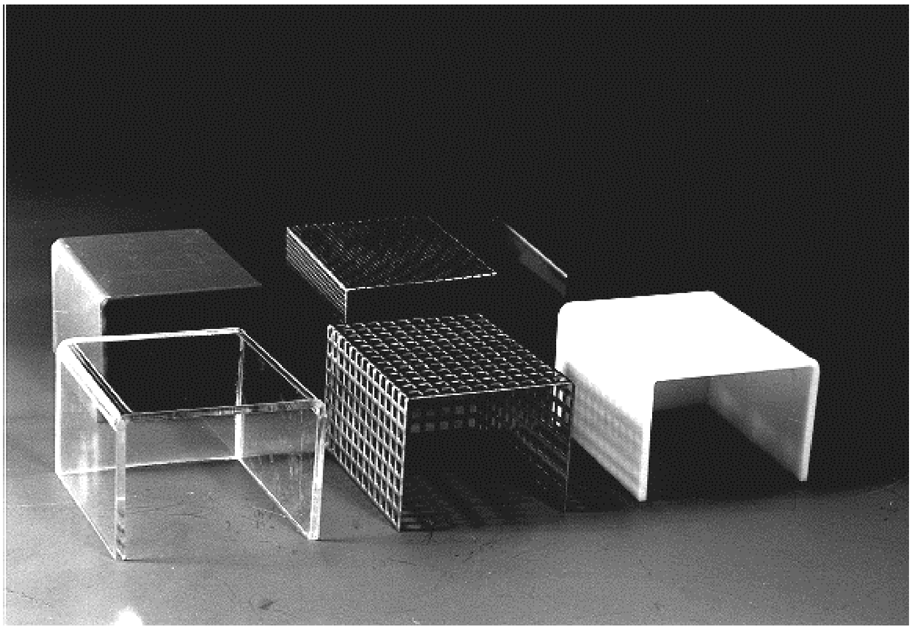

Figure 1 *The six different nest boxes tested in the preference test. Bottom row (light series): clear perspex (left), metal grid (middle), white perspex with holes (right). Top row (dark series): grey PVC (left), perforated sheet metal (middle), sheet metal (right).*

In the first series, three nest boxes were tested, made of materials which where rather translucent (light series). They were made of clear perspex, metal grid and white perspex with three holes in each side wall, two in the back wall and nine holes in the roof (diameter of holes: 5 mm). In the second series three nest boxes were tested, made of more opaque materials (dark series). They were made of grey PVC, perforated sheet metal and sheet metal. In both series, the fourth cage contained only sawdust bedding. In the third series, per strain and sex the nest boxes which most animals had chosen in the first two series were tested in a two-cage system.

In a fourth test series the perforated metal nest box (closed) was tested against a similar nest box which had the rear wall removed (open), to test whether the mice preferred an open or closed nest box. For this purpose a two-cage test system was used.

During the series with the light nest boxes the behaviour of one animal was recorded for 12 h during day time (second day of the test) and for 12 h during night time (second night of the test). The behaviour of the mice during a series with dark nest boxes was not recorded, because it was not possible to observe a mouse when it was in a dark nest box.

Statistical analysis

The dwelling time data were analysed by distinguishing three time frames: the total of dwelling times during the 48 h of the experiment, the dwelling times during 12 h of day light (second day of the test) and the dwelling times of 12 h of night time (second night of the test). These two latter periods synchronised the periods of collected behavioural data (video tape recordings).

The method of statistical analysis used has been described by Blom et al (1995). Briefly, per test series the dwelling time data (in seconds) were logarithmically transformed as they were not always normally distributed, and to increase the independence of the data. For the same reason, central cage dwelling times were not included in the analysis. Food and water intake data were not transformed, because they were normally distributed.

The data were analysed using multivariate repeated measures analysis (Wilk's lambda) to evaluate the influence of type of nest box and interactions on choice behaviour and to detect possible differences between the strains or the sexes of a strain in choice behaviour. Food and water intake were analysed in a similar way as the dwelling times. Statistical significance was pre-set at $P<0.05$.

Overall significant differences in dwelling times and amount of food and water consumed between choice cages were further analysed using paired t-tests to indicate which of the cages were preferred or avoided. As multiple comparisons were made, the level of statistical significance was pre-set at $P<0.0083$ (Bonferroni's adaptation).

Behavioural data

The behavioural data on video tape were viewed and analysed using a behavioural observation software package (The Observer v 2.0, Noldus BV, The Netherlands). The tapes were viewed at normal speed, thus behaviour was seen nine times faster than the original behaviour. Every 5 s the behaviour was scored, which corresponds to one sample every 45 s in reality. The following ethogram was used to classify the behaviour (based upon Blom et al 1992):

Sleeping in a nest box (sl-in) =

movements are absent while the animal is in a sitting or lying position. Very short or minor movements during a long resting period (eg turning) are not considered as an interruption.

Sleeping outside a nest box (sl-out) =

same as previous, except that the behaviour is performed outside a nest box.

Grooming in nest box (gr-in) =

while sitting or standing, the mouse is shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals.

Grooming outside a nest box (gr-out) =

same as grooming in a nest box, except performed outside a nest box.

Ingestive behaviour (ing) =

includes eating and drinking behaviour. Eating: gnawing on food particles from the food hopper or from the sawdust, coprophagy is included as well. Drinking: licking the nipple of the drinking bottle.

Exploration in a nest box (ex-in) =

this includes all locomotion (movements) and digging (pushing bedding material forwards or backwards with nose, fore paws or hind legs) performed in a nest box.

Exploration outside a nest box (ex-out) =

locomotion, rearing (standing on hind feet, fore paws not touching the floor) and digging performed outside a nest box.

Exploration on a nest box (ex-on) =

locomotion and rearing on a nest box

Climbing (clim) =

climbing on or hanging from the bars of the wire cage lid or food hopper, or standing on the passage tube or drinking nipple. While climbing or hanging the hind legs or tail may touch the cage walls.

Descriptive statistics were used to analyse the behavioural data, because only two animals from each sex and strain group (N=12) were observed in the test series with the light nest boxes. The distribution of behaviour in each test cage was analysed for the night and day time period separately.

RESULTS

Cage choice

In the series with light and dark nest boxes, significant cage choices were made by mice of both strains during all three time periods (all P<0.05), except for the BALB/c mice during the night period in the dark nest boxes series.

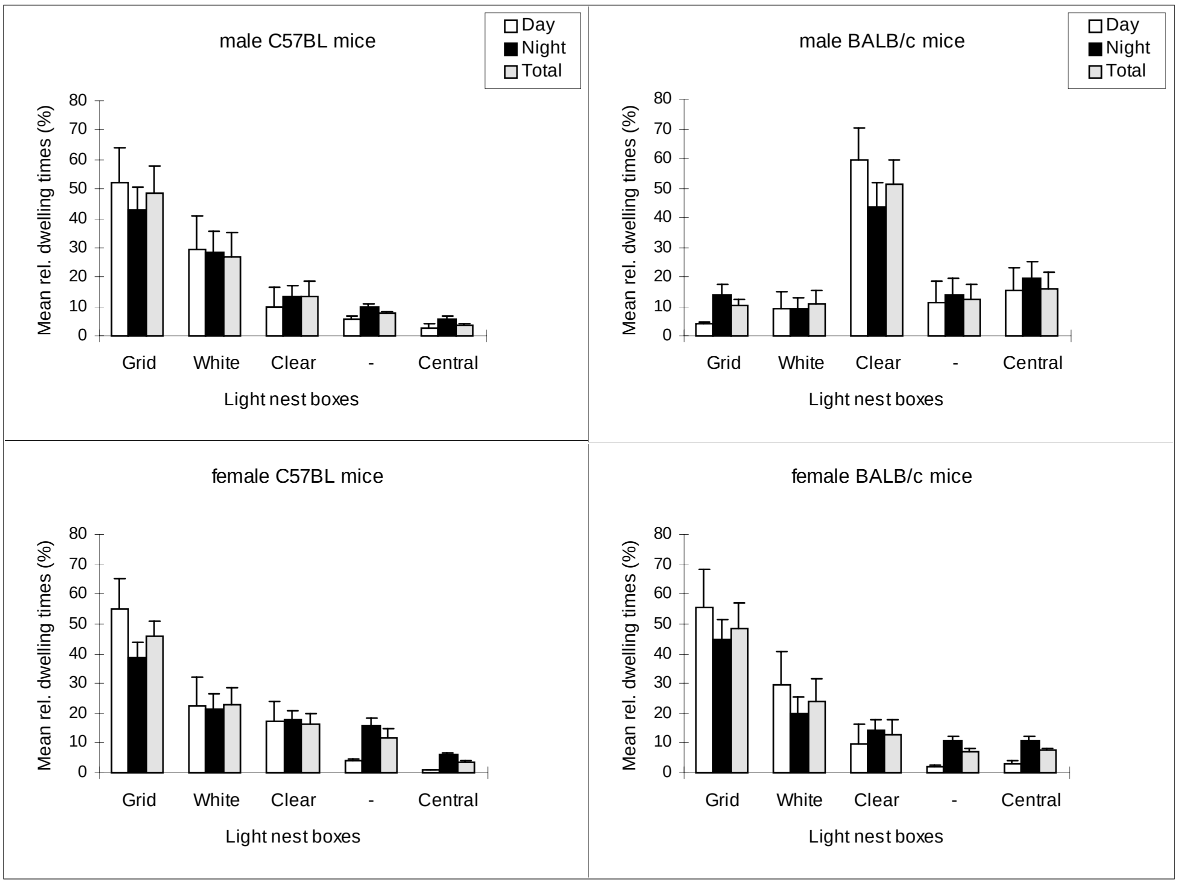

Figure 2 *Results of the preference test with three nest boxes made of light materials. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for mice of two strains (N=47).*

Figure 2 shows the mean relative dwelling times (and SEM) per cage for the series with the light nest boxes. C57BL mice preferred the metal grid nest box (no significant difference between the sexes). Main contrasts found for the C57BL mice were between the cage with the metal grid nest box on the one hand and the clear perspex nest box or cage without a nest box on the other (paired t-tests, all P<0.005, all three time periods). BALB/c males preferred the clear perspex nest box, BALB/c females the metal grid nest box (significant difference between the sexes in all three time periods, MANOVA, all P<0.001). Main contrasts for both sexes were found to exist between the cages with the metal grid nest box and the clear perspex nest box (paired t-tests, all P<0.01, all three time periods). For the BALB/c females significant contrasts were also found between the cage without a nest box and the cage with the metal grid nest box (paired t-tests, all P<0.001, all time periods), and for the BALB/c males between the cage with the clear perspex nest box on the one hand and the cage without a nest box or the cage with the white perspex nest box on the other (paired t-test, all P<0.01, all time periods). In this series the difference between the strains appeared to be significant during total and night time period (MANOVA, both P<0.05).

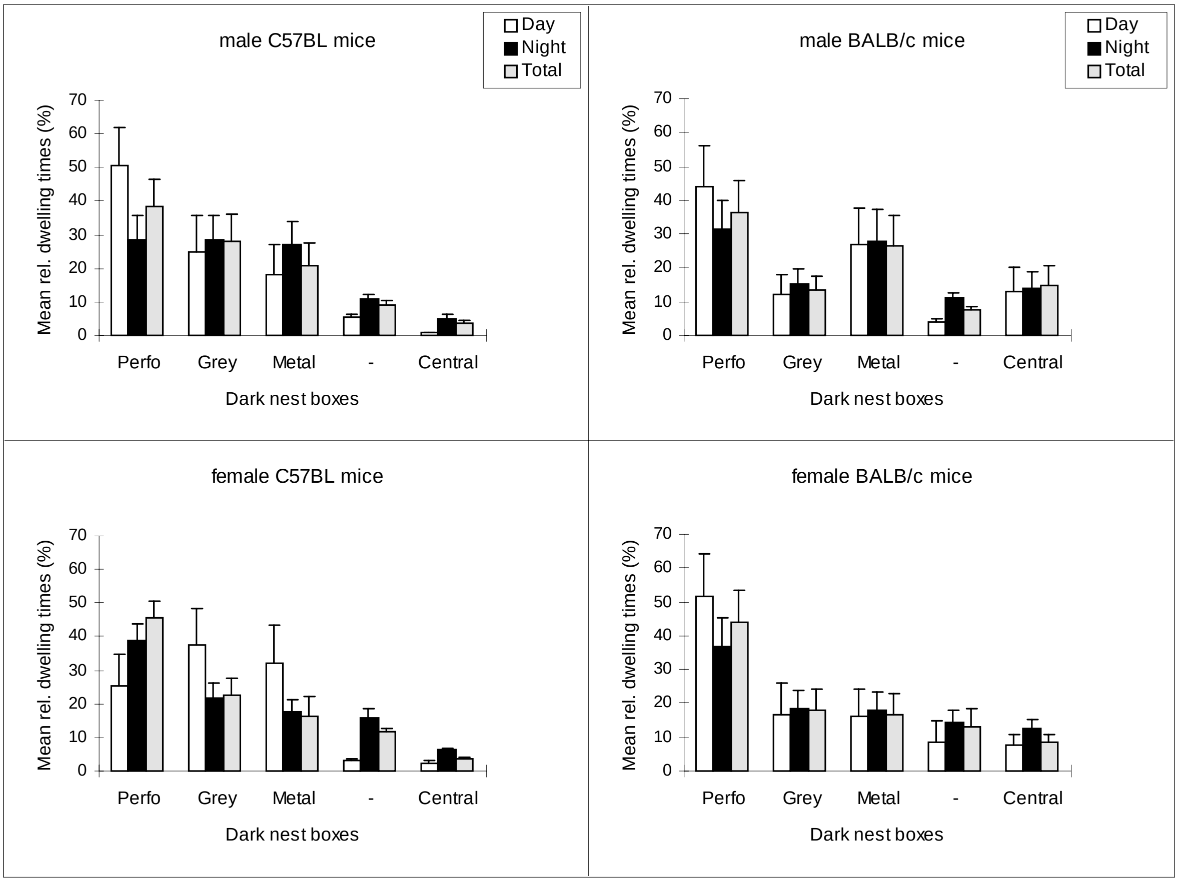

Figure 3 *Results of the preference test with three nest boxes made of dark materials. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for mice of two strains (N=47).*

Figure 3 shows the mean relative dwelling times (and SEM) per cage for the series with the dark nest boxes. C57BL mice chose the perforated metal nest box (no significant difference between the sexes). Significant contrasts were found for almost all time periods between the cage without a nest box on the one hand and the cages with the perforated metal, grey PVC or sheet metal nest box on the other (paired t-tests, all P<0.005). BALB/c mice preferred the perforated metal nest box (no significant difference between the sexes). The only significant contrast here was found between the cage with the perforated metal nest box and the cage without a nest box, but only for the total time period (paired t-test, P<0.001). In this series there was no significant difference between the strains in cage preference.

In the light vs dark series the nest boxes which most mice had chosen in the previous two series were tested against each other, being the perforated metal nest box vs the metal grid nest box for the C57BL mice and BALB/c female mice, and the perforated metal nest box vs the clear perspex one for the BALB/c male mice. No significant cage choices were made by both strains during all time periods (results not shown).

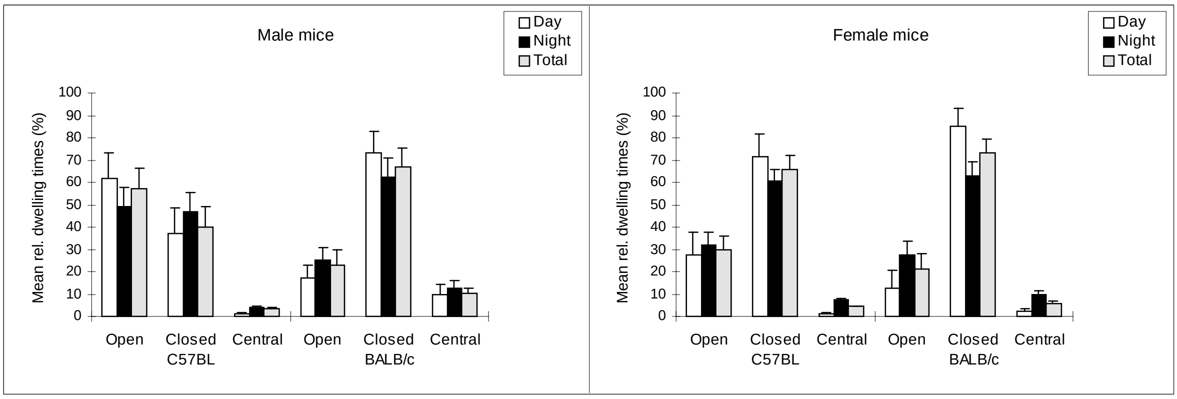

Figure 4 *Results of the preference test with the open (two open sides) vs closed (one open side) nest box. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for mice of two strains (N=47).*

In the open vs closed nest box series (Figure 4) the BALB/c mice significantly chose for the closed nest box during all three time periods (MANOVA, all P<0.005). In the C57BL strain only the females made a significant choice for the closed nest box during the total time period (MANOVA, P<0.05, difference between the sexes, MANOVA, P<0.05). There was a significant difference between the strains in this series during the total and day time period (MANOVA, all P<0.05).

Food and water intake

In all four test series no significant cage choices for food intake were made by either strain. Significant cage choices for water consumption were made in the series with light nest boxes. C57BL mice drank most in the cages with the white perspex nest box and the cage without a nest box. (MANOVA, P<0.05). BALB/c mice drank most in the cage without a nest box (MANOVA, P<0.005). There were no differences between the sexes in both strains. However, the difference between the strains was significant (MANOVA, P<0.05). Significant contrasts for water intake were found for the BALB/c mice between the cage without a nest box on the one hand and the cages with the white or clear perspex nest box on the other hand (paired t-tests, all P<0.005).

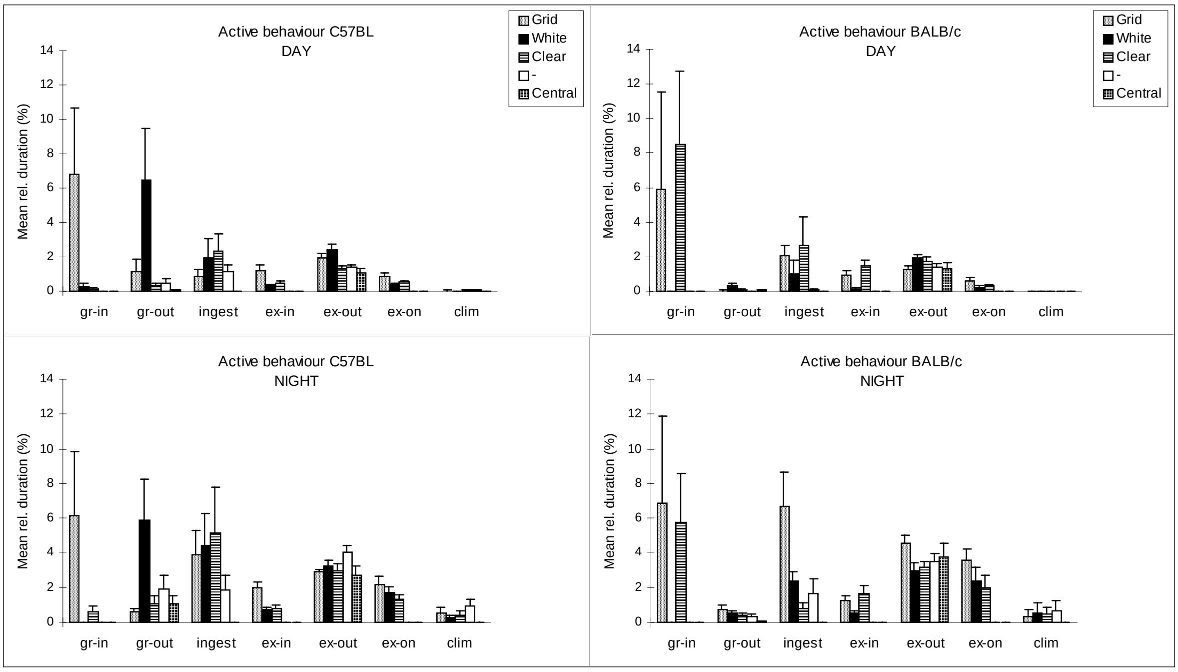

Figure 5 *Results of behavioural observations from preference tests with light nest boxes with mice of two strains (N=7). Mean relative time (and SEM) spent on indicated active behaviour during day (=12 h) and night (=12 h) period (See Animals and Methods for explanation of abbreviations).*

In the series with dark nest boxes BALB/c mice (no significant difference between the sexes) drank most in the cage without a nest box (MANOVA, P<0.05), the contrasts between the cage without a nest box and the cages with the grey PVC or sheet metal nest box were significant (paired t-test, both P<0.005). In the light vs dark nest box series and in the open vs closed series no significant cage choices for water consumption were made by either strain.

Behavioural data

Figure 5 illustrates the distribution of active behaviour during day and night periods for BALB/c and C57BL mice in the series with the light nest boxes. Figure 6 shows sleeping behaviour during day and night periods. Behavioural categories are indicated as relative durations of the total amount of behaviour performed. Behavioural data of one BALB/c male mouse is left out, because it preferred the central cage for sleeping. As this was the only mouse showing this preference its behaviour was not considered representative for the behaviour of the other mice in this test series.

During day time the mice mostly slept in their preferred cage in the nest box, but some C57BL mice sometimes slept in front of the nest box (sl-out). During the night the mice were active and showed exploration, climbing and ingestive behaviour in all four cages of the test system, although also a fair amount of time was spent on sleeping.

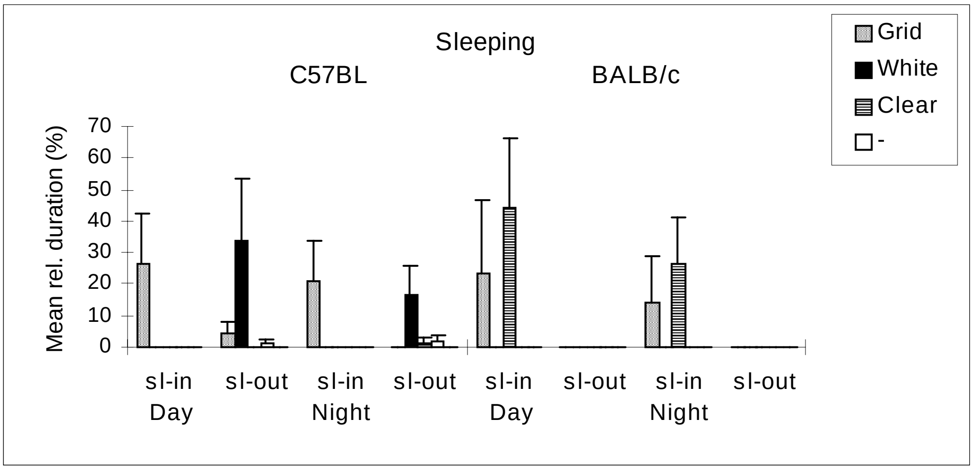

Figure 6 *Results of behavioural observations from preference tests with light nest boxes with mice of two strains (N=7). Mean relative time (and SEM) spent on sleeping behaviour during day (=12 h) and night (=12 h) period (See Animals and Methods for explanation of abbreviations).*

DISCUSSION

The results of this study show that the mice do make choices between different cages and that they prefer a cage containing a nest box. In general, the preferred nest boxes were those consisting of perforated metal or grid metal. These results are comparable with the results obtained by Buhot (1981, 1986, 1987, 1989) who used a different approach. In her study mice had to choose between six nest boxes in six successive trials. Within a trial the nest boxes were identical in design but different in material, whereas between trials different designs were used (each nest box had only walls but no roof). Mice appeared to make their choices on the basis of the materials and not on the basis of the design. The preferred materials in Buhot's study were the darker ones: close grid and perforated metal. These materials are comparable with the perforated metal and grid metal in our study. Both metal structures allow olfactory cues to pass, but are difficult to look through; however, some light may penetrate. Buhot concluded that visual cues may have been of most importance in nest box choice and that the mice used the same criterion (darkness) to make a choice. Hamsters also chose a dark nest box as nest site, the degree of illumination of the nest box was the criterion that prevailed over the size of the nest box and the distance to a nesting material dispenser, but only two types of nest boxes - clear or black - were offered (Ottoni & Ades 1991).

In our test series with the dark nest boxes, however, the mice did not prefer the darkest nest boxes (sheet metal or grey PVC), but chose the perforated metal nest box. In the series with light nest boxes the BALB/c male mice preferred the clear perspex nest box, which let most light through, compared with the others. This choice is difficult to explain if one expects mice to choose a dark nest box as a shelter against too much light. BALB/c mice are albinos and therefore their eyes are sensitive to retinal damage caused by high light intensities (Clough 1987). We also observed that in the series with the light nest boxes some C57BL mice (two males, seven females) did not always occupy the nest box, but sometimes slept in front of it. The behavioural graph (Figure 6) illustrates this behaviour for the C57BL strain. Apparently, these mice did not use the nest box to hide for light either, but C57BL mice have pigmented eyes and are therefore less sensitive than BALB/c mice for high light intensities. But when the mice were disturbed they always ran into the nest box for hiding. Schleidt (1951) described similar behaviour, he observed mice sleeping in front of glass tubes which were too small to sleep in, but when they were alarmed they fled into the tubes.

The results suggest that not only visual cues determine the choice of the mice for a nest box, but perhaps also olfactory cues play a role. Most mice chose nest boxes which were not made of completely closed material, but contained holes in the walls (perforated metal and grid metal), which allow olfactory cues to pass. Odours play an important role in social behaviour of mice, especially in identifying individuals and their dominance status (Rawleigh et al 1993). Schleidt (1951) describes that wild house mice and field mice use different cues when selecting an object, depending on whether they choose a shelter or a nesting site. For the choice of nest site tactile cues are most important, whereas for a shelter light intensity cues are most relevant. Mackintosh (1973) has shown that in large enclosures male mice appear to use visual cues for the detection of territorial boundaries. More research is needed to find out what under various environmental conditions, the most important criteria for nest box choices are.

In the experiments by Buhot-Averseng (1981) the animals showed a high agreement in nest box choice. However, in our study, although overall choices were significant for the groups, individual mice did not always made a clear choice for one nest box, but spent time in two nest boxes (0-5 animals per group). The incidence of this behaviour was highest in the group of female C57BL mice. In a field study on the utilisation of nest boxes by rodents Truszkowski (1974) observed that adult rodents (except females rearing young) usually use a large number of shelters simultaneously. The experimental set-up of Buhot-Averseng did not allow the mice to choose more than one nest box, because after two hours of time spent in one nest box it was removed.

The behavioural diagram in Figure 5 shows that climbing on the cage lid does not occur very often during day or night, although in standard cages it is a major and regularly occurring component of locomotor activity (Büttner 1991). When comparing Figure 5 with similar patterns obtained from observations of mice in preference tests with nesting material (Van de Weerd et al accepted/a), it can be seen that climbing has a much higher frequency. It seems that in the present study sitting on the nest box (in Figure 5: exploration on a nest box) partly substitutes climbing on the cage lid, so the nest box also serves as a climbing object.

The results of test series with the open vs closed nest box show that most mice preferred the closed nest box with only one open side, which seems to contradict the observation that they normally build burrows with more openings (Adams & Boice 1981; Dudek et al 1983). The structure of the nest boxes, however, did not allow the mice to control both openings when lying inside, so this may have been the reason that they preferred the nest box with only one open side. The animals were also observed to lie at the rear end of the nest box with their heads directed towards the opening. Mice provided with bottles as refuges showed similar behaviour, they tended to sleep with their heads toward the bottle opening (Ward & DeMille 1991).

Although the results of this study do not clearly indicate which criteria mice use to choose a nest box, it is clear that individual mice prefer a cage with a nest box and avoid cages without one. It is important, however, to consider the structure of the nest boxes when using them as enrichment. Slight modifications in the enriched environment can lead to unpredictable effects on mice (Bergmann et al 1994/1995). Several authors have reported increased aggression when applying a structure offering shelter in the home cage of group housed mice. Haemisch & Gärtner (1994) and Haemisch et al (1994) used a horizontal labyrinth consisting of vertical PVC dividers in a standard cage. In these enriched cages they observed an increase in intermale aggression when introducing unfamiliar males and a less stable social structure. The authors suggested that the available structures enhanced the territorial tendencies of the male mice. Similar effects on aggression were reported by Bergmann et al (1994/1995) who used a cage insert consisting of a passage-way, comparable with the structure used by Haemisch & Gärtner (1994) and Haemisch et al (1994). This structure led to an increase in aggression, measured as number of bite wounds.

Other authors have used shelter-like structures which did not elicit a higher incidence of aggression. Chamove (1989b) used an insert which created corridors and Ward & DeMille (1991) used plastic bottles as shelters. In a study by Rawleigh & Kemble (1992) the provision of a nest box decreased offensive behaviour in a resident-intruder test. Differences between these studies may be partly explained by the different strains of mice used, as some strains are reported to be more aggressive (Mondragón et al 1987) and it is likely that these strains are more sensitive for effects of enrichment. Haemisch & Gärtner (1994) also reported differences in the level of aggression between the two strains used (DBA/2J and CBA/J).

The results of the above mentioned studies, also suggest that the structure of the insert might be a determining factor in stimulating aggression. Inserts in the cage structure the living environment and this may have an influence on the hierarchical system which normally develops when male mice are kept in a group (Haemisch et al 1994; Mackintosh 1981). Structures with only a few openings do not resemble natural settings. When given wild mice and laboratory mice the opportunity to burrow, their burrows contain al lot of openings to the surface, so there always are several routes for fleeing (Adams & Boice 1981; Dudek et al 1983). This was impossible in the designs used by Haemisch et al (1994), Haemisch & Gärtner (1994), and Bergmann et al (1994/1995), which may have given dominant males the opportunity to defend the few entrances into the corridors and this may have led to social instability. Bergmann et al (1994/1995) used a second passage way with more openings as well, which did not increase the number of bite wounds as much as the structure with only two openings. It is therefore important to study the effects of the application of a shelter for enrichment purposes (taking into account the structure and design), before applying it on a larger scale (Van de Weerd & Baumans 1995). All mice in this study preferred a cage with a nest box, which implies that nest boxes may be used for enrichment purposes.

Acknowledgements
The authors like to thank NA Miltenburg for construction of the nest boxes.