# STRENGTH OF PREFERENCE FOR NESTING MATERIAL AS ENVIRONMENTAL ENRICHMENT FOR LABORATORY MICE

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**SUMMARY**

*The present paper describes two experiments in which preferences of laboratory mice for materials which could serve as cage enrichment are investigated. Previous research revealed clear preferences for a cage with nesting material or a nest box instead of a cage with only bedding material. Both enrichment items may offer a hiding place e.g. as a means to avoid aggressive cage mates or over-exposure to light.*

*In the first experiment, the most preferred nesting material and the most preferred nest box (results from previous studies) were tested against each other. A strong preference of all mice was found for the nesting material. In the second experiment, the preferred nesting material was combined with a grid floor (previously found to be avoided) and the nest box was combined with bedding material. All mice preferred the cage with the nesting material. The mice were highly motivated to use nesting material, despite the presence of a grid floor. Thus it is concluded that providing a cage with nesting material may have a positive effect on the well-being of laboratory mice.*

## INTRODUCTION

Environments of laboratory animals have often been designed on the basis of economic and ergonomic aspects, with little or no consideration for animal welfare. Laboratory housing conditions can deprive animals of the possibility to perform a full repertoire of normal behaviour (Van de Weerd & Baumans 1995). The inability to engage in species-specific behaviour may cause signs of suffering such as abnormal behaviour or pathology (Jensen & Toates 1993). Environmental enrichment alters the environment by introducing materials or objects which are stimulating for the animals and which allow them to express more of their natural behavioural repertoire, thereby enhancing their well-being. Different animal species may have different enrichment requirements and when introducing enrichment to an animal's environment it is very important to evaluate whether or not the animal responds to the enrichment. Preference tests can be used to determine some general principles about species-relevant properties of enrichment devices (Mench 1994).

Previous studies on the preferences of laboratory mice for items which could serve as enrichment, revealed clear preferences for a cage with nesting material or a nest box instead of a cage with only bedding material (Van de Weerd et al submitted/a; accepted/a). Nesting material may have several functions. By building a nest mice can regulate their temperature and avoid too much light or hide for aggressive cage mates. Nest boxes may provide a shelter or refuge because they give mice the opportunity to actively withdraw from frightening stimuli inside or outside their cage (Van de Weerd and Baumans 1995).

The present paper describes two experiments in which these preferences are further investigated. The first experiment investigates if mice prefer a nest box over nesting material or vice versa by testing the most preferred nesting material and the most preferred nest box from both previous studies against each other. This experiment showed a strong preference of all mice for the nesting material. Therefore, in a second experiment the importance or strength of the preference for nesting material was studied.

One general criticism on preference tests is that they only give information about the relative properties of the choices given, but do not indicate the importance an animal attaches to a preferred option. In order to interpret the results of preference tests and to be able to apply them to practical situations where an improvement in welfare is sought, the strength of the preferences should be established (Broom 1988; Broom & Johnson 1993; Dawkins 1983; Duncan 1992; Fraser 1996). Where animals show that they are willing to work hard for the choices offered it is reasonable to conclude that their welfare is improved by achieving that objective (Broom 1988).

Several methods have been developed to measure the strength of preferences (see also Sherwin & Nicol 1995), e.g. the instrumental or operant technique approach, where an animal has to learn to activate some mechanisms such as lever pressing or lifting a weighted door (Collier et al 1990; Duncan 1992; Manser et al 1996; Roper 1973) or the natural obstacle or obstructive techniques approach where an animal has to overcome a natural barrier such as a narrow gap or water (Duncan 1992; Sherwin & Nicol 1995). An animal may however, not always be able to learn an operant response (Duncan 1992), it is therefore important that they associate the required activities with the goals to be reached and that the behaviour required for expressing the preference is reasonably natural for the type of reward (Fraser 1996). Behaviours such as lever pressing or lifting a weight are not very natural for most animals.

In experiment 2 of this study we have adopted the method of balancing one preference against another, as previously used by e.g. Van Rooijen with gilts (1980), Dawkins with hens (1981, 1983) and Blom et al with mice (1993). The testing variables (nesting material and nest box) were balanced against cage floor covering. Previous preference tests with the same strains of mice, showed that mice preferred bedding material and avoided wire mesh as floor covering (Blom et al 1996b). Thus the preferred nesting material (Van de Weerd et al accepted/a) was combined with the previously avoided grid floor and the preferred nest box (Van de Weerd et al submitted/a) was combined with previously preferred bedding material. This approach will give an indication whether the mice are willing to accept a grid floor in order to use the nesting material or whether the combination of sawdust with the nest box is more attractive.

## ANIMALS AND METHODS

### EXPERIMENT 1

*Animals*

Female and male mice of two strains (C57BL/6JIcoU and BALB/c AnCrRyCpbRivU, N=47) were used. The experiment was conducted in two cohorts. At the start of the first cohort (females, n=24) the mice were 13-14 weeks of age. At the start of the second cohort (males, n=23) the mice were 30-31 weeks of age. One male BALB/c mouse died before the experiments started. Both groups of mice were familiar with the nesting material and nest box offered in the test series (either in previous preference test series or in their home cages).

*Housing*

The animals were housed (per strain and sex) in groups of six animals 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 to allow the mice to get used to them. Both cages were supplied with food pellets *ad libitum* (RMH-B, Hope Farms, Woerden, The Netherlands), tap water *ad libitum* and sawdust bedding (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen Holzmühle, Germany). The animals were kept in conventional rooms with controlled photo period (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 two 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). 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 mice.

The test cages were supplied with 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. The signals were send to a computer which calculated 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. Both test cages and the central one, were 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 had red lights (approx. 5 lux at 1 m) to enable video recordings.

*Procedure*

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. 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). Food and water of each test cage were weighed before and after the experiment.

Per strain and sex group the most preferred nesting material (Van de Weerd et al accepted/a) was tested versus the most preferred nest box (Van de Weerd et al submitted/a). Both items were offered in a test cage supplied with 50 g of sawdust bedding (Lignocel 3/4). Table 1 shows the materials tested.

***Table 1*** *Materials tested in experiment 1*

| Animals | Nesting material (amount) | Nest box (8x10x6 cm) |
|---|---|---|
| *C57BL* | | |
| males | Paper towel[1] (1 piece) | perforated metal |
| females | Kleenex® tissues[2] (2 pieces) | perforated metal |
| *BALB/c* | | |
| males | Kleenex® tissues[2] (2 pieces) | clear perspex |
| females | Kleenex® tissues[2] (2 pieces) | perforated metal |

[1] Celtona, Cuijk, The Netherlands
[2] Kimberly-Clark Corporation®, EEC

## EXPERIMENT 2

*Animals*

Previous experiments with preferences for nesting materials and nest boxes revealed no major differences in preferences between the sexes of a strain, therefore in experiment 2 only female mice were used. The same female mice of experiment 1 were used in experiment 2 (C57BL/6JIcoU and BALB/c AnCrRyCpbRivU, N=24). At the start of experiment they were 16-17 weeks of age.

Housing conditions, test system and behavioural observations were similar as described for experiment 1.

*Procedure*

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

The perforated metal nest box of experiment 1 was offered in a test cage with 50 g of sawdust bedding (Lignocel 3/4) and was tested against nesting material (Kleenex tissues, 2 pieces). The nesting material was offered in a cage with an inserted wire grid floor (stainless steel wire, rod diameter 2 mm, mesh size 10x10 $mm^2$). See Figure 1.

*Statistical analysis (experiment 1 and 2)*

The dwelling 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 with 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. Data on food and water intake were not transformed, because they were normally distributed.

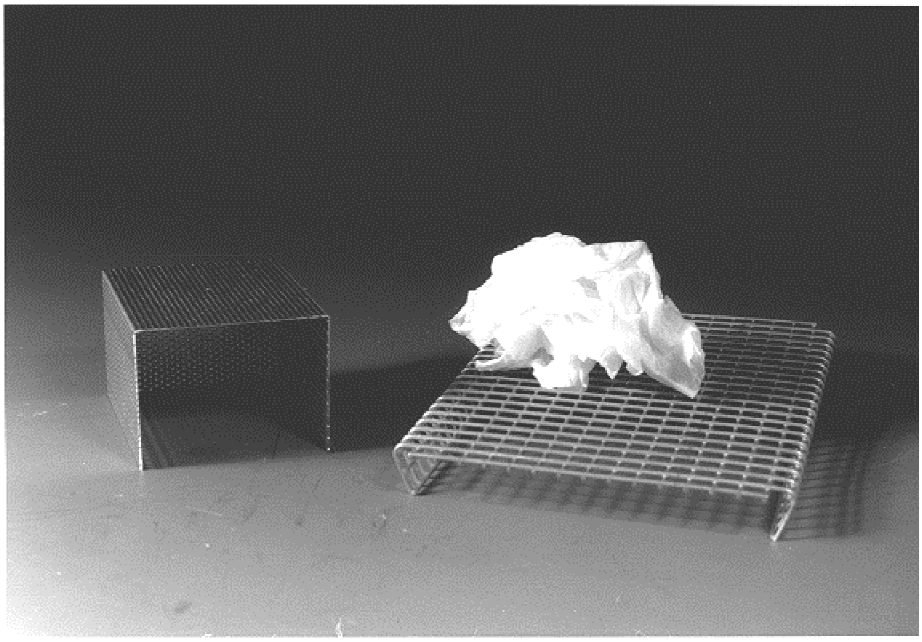

***Figure 1*** *Materials tested in experiment 2. Left: perforated metal nest box. Right: grid floor with two Kleenex tissues.*

The data were analysed using paired t-tests to evaluate the influence of cage contents on choice behaviour and to detect possible differences in choice behaviour. Food and water intake were analysed in a similar way as the dwelling times. Statistical significance was pre-set at $P<0.05$.

*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 nest box or nesting material (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 (e.g. turning) are not considered as an interruption.

Sleeping outside nest box or nesting material (sl-out) =

same as sleeping in, except the behaviour is performed outside nest box or nesting material.

Grooming in nest box or nesting material (gr-in) =

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

Grooming outside nest box or nesting material (gr-out) =

same as grooming in, except performed outside nest box or nesting material.

Manipulation (man) =

manipulation of the nesting material (shredding, fraying, dragging and nest building behaviour) or nest box (pushing, pulling, gnawing).

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 nest box or nesting material (ex-in) =

this includes all locomotion (movements), rearing (standing on hind feet, fore paws not touching the floor) and digging (pushing bedding material forwards or backwards with nose, fore paws or hind legs) performed in nest box or nesting material.

Exploration outside nest box or nesting material (ex-out) =

locomotion, rearing and digging performed outside nest box or nesting material.

Exploration on 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 per test series. The results were used to describe the behaviour of the mice in the different test cages during a test series.

## RESULTS

*Experiment 1*

Figure 2 illustrates the mean relative dwelling times per cage for experiment 1.

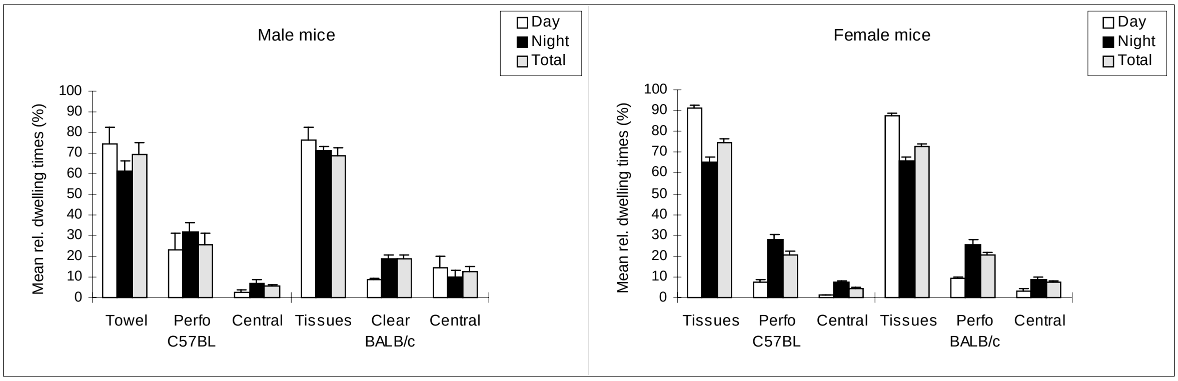


***Figure 2*** *Results of the preference test with nesting materials and nest boxes. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for male and female mice of two strains (N=47).*

All mice spent significantly more time in the cage with the nesting material during all three time periods (C57BL male mice: paper towel, all P<0.05, other groups: tissues, all P<0.001). BALB/c males spent significantly more time in the cage with the (clear perspex) nest box to eat (P<0.05), the other groups did not have a preferred cage for food intake. Female mice of both strains preferred the cage with the nesting material (tissues) for drinking (both P<0.05).

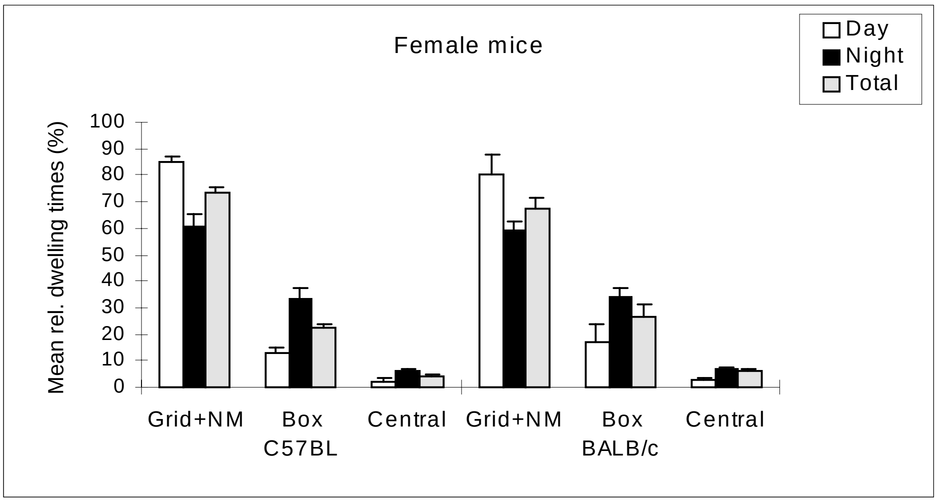


***Figure 3*** *Results of the preference test with nesting material provided on a grid floor and a nest box on bedding material. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for female mice of two strains (N=24).*

*Experiment 2*

Figure 3 illustrates the mean relative dwelling times per cage for experiment 2. Female mice of both strains preferred the cage with the nesting material on the grid floor during all three time periods (all P<0.01). They preferred the cage with the nesting material (tissues) on the grid floor to drink (both P<0.05), but had no preferred cage for eating.

*Behavioural data*

Figure 4 illustrates the distribution of active behaviour (no sleeping) for BALB/c and C57BL mice in experiment 1 and 2. Only the night data are shown, because day time data had similar patterns in both series. A lot of grooming outside the nest box (gr-out) is performed in both experiments, but grooming outside the nesting material is mostly seen in experiment 1 and not in experiment 2 where the nesting material is provided on the grid floor.

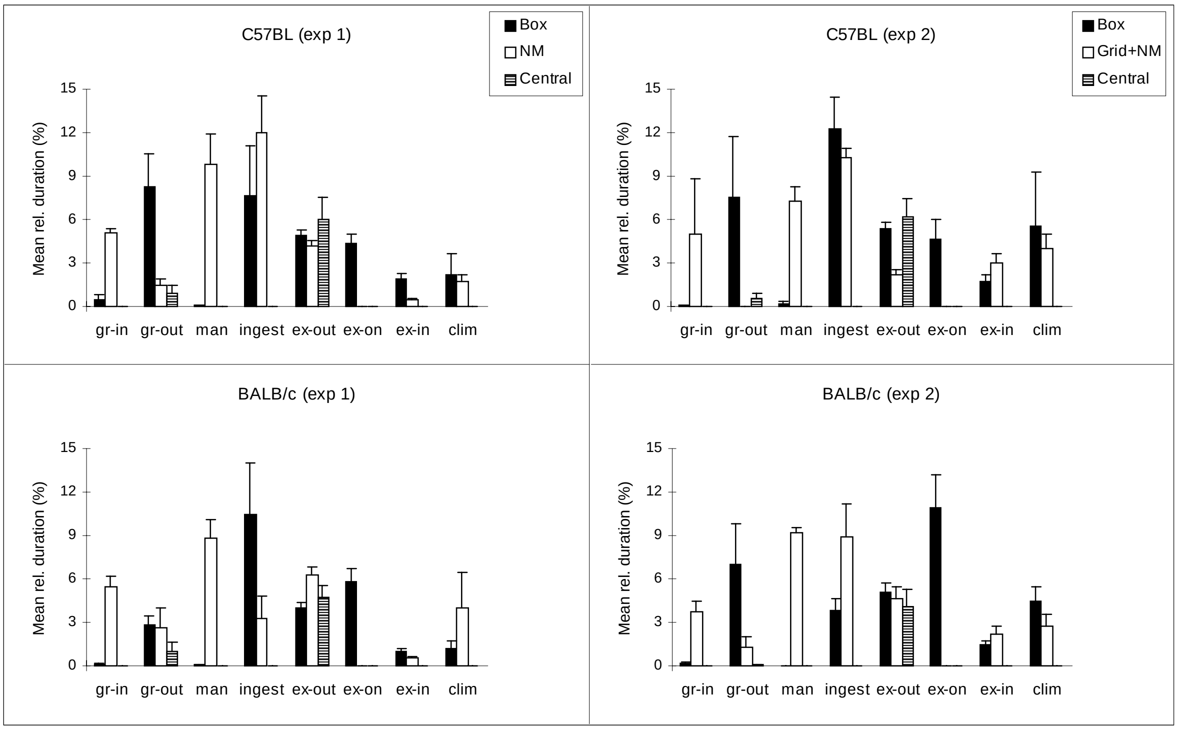


***Figure 4** Mean relative time (and SEM) spent on indicated active behaviour during the night time period (12 h). Left: experiment 1, preference tests with nesting material vs a nest box (male and female mice of two strains, N=8). Right: experiment 2, preference tests with nesting material on a grid floor vs a nest box on bedding material (female mice of two strains, N=4). (See Animals and Methods for explanation of abbreviations).*

Figure 5 shows the distribution of sleeping behaviour during the night in experiment 1 and 2. Most sleeping was performed in the nesting material (sl-in). When comparing the night time behavioural patterns from both series, it can be seen that in experiment 2 the mice show more sleeping in the cage with the nest box, although they do not lie inside the nest box (sl-out), this is observed to a much lesser extent in experiment 1, with the nesting material provided on bedding.

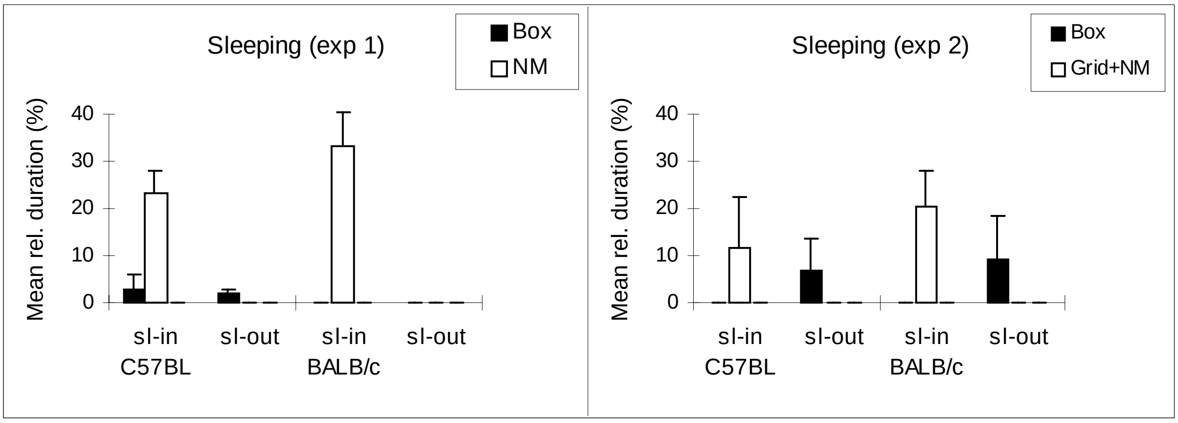


***Figure 5*** *Mean relative time (and SEM) spent on sleeping behaviour during the night time period (12 h). Left: experiment 1, preference tests with nesting material vs a nest box (male and female mice of two strains, N=8). Right: experiment 2, preference tests with nesting material on a grid floor vs a nest box on bedding material (female mice of two strains, N=4). (See Animals and Methods for explanation of abbreviations).*

## DISCUSSION

All groups of mice showed a clear preference for the nesting material in experiment 1, with 60-90 % of the time spent in the cage with the nesting material. Cages with nest boxes were mainly visited during the night, when the mice were active and explored the test system (see Figure 4). In previous preference tests with different types of nest boxes, mice did prefer a cage with a nest box to one with no nest box (Van de Weerd et al submitted/a), but in the present experiment the nesting material appeared to be more attractive. This was also shown clearly in experiment 2, where again, all mice had a strong preference for the cage with the nesting material although a grid floor was present. Previous studies have shown that rodents avoid grid floors when alternatives are offered (Arnold & Estep 1994; Blom et al 1996a, 1996b; Manser et al 1995, 1996; Van de Weerd et al 1996).

In previous preference tests with nesting material, approximately half the number of (naive) mice made a combination of the most preferred nesting materials by dragging them from one cage to another (Van de Weerd et al accepted/a). In the present study this behaviour was not seen. In both experiments all mice spent most time in the cage with the nesting material and even in experiment 2 the mice did not drag the nesting material to the cage with the nest box to combine both commodities or at least lie in the bedding. It can be argued that they accepted the grid floor in order to rest in the nesting material, as the nesting material masks the structure of a grid floor. Figure 6 shows the type of nests the mice made on the grid floors, which have the same shape as nests constructed on bedding.

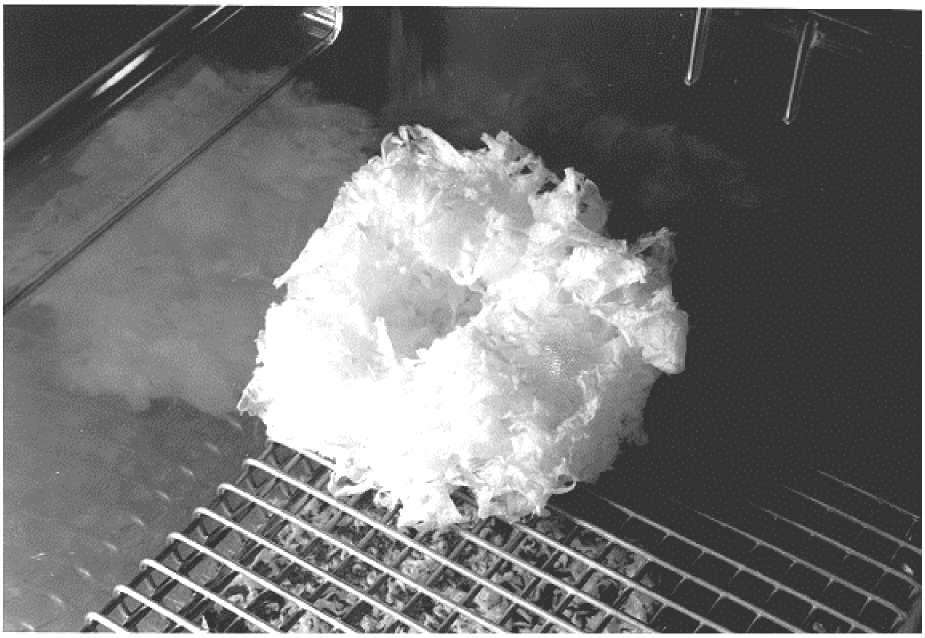

***Figure 6*** *Example of a nest built on the grid floor (experiment 2).*

These results imply that nesting materials are much more attractive for mice than nest boxes. In natural settings nest boxes may be used by rodents as a feeding post, as a storage for food, for the construction of a nest or for the bearing and raising of offspring as described by Ryszkowski & Truszkowski (1970). They may also offer an opportunity to hide from predators. In the laboratory the function of a nest box is more restricted, the main function probably is to offer a shelter for overexposure to light or to avoid aggressive cage mates (Van de Weerd & Baumans 1995). Nesting material has similar functions, but differs from nest boxes in that it can be manipulated to build a nest and by doing this, the mice are able to structure their environment (Van de Weerd et al accepted/a). Another main function of a nest is to shelter animals from variations in environmental temperatures (Brain & Rajendram 1986). Both males and females will build a nest when offered nesting materials and there is a strong genetic influence on nest building behaviour (Brain & Rajendram 1986; Lee 1972, 1973; Lisk et al 1969; Lynch & Hegmann 1972).

Several studies have shown that mice are willing to work in order to get nesting material, e.g. when nesting material is put on the cage lid, they start pulling it into the cage (Lisk et al 1969; Lynch & Hegmann 1972; Wolfe & Barnett 1977). Roper (1973) showed that mice can be trained to press a key in order to obtain nesting material (paper strips). The paper acted as a reinforcer for this response. Collier et al (1990) described a similar experiment with rats, which were motivated to press a response bar often in order to reach a nest, although they were willing to press more in order to reach food or water. This phenomenon has also been described in other studies, which compared the demand for certain behavioural activities with the demand for food or water (Dawkins 1983; Matthews 1994; see also discussion in Roper 1973). In general, animals are willing to work harder for food or water than for other commodities. This is not surprising, because animals will almost always be highly motivated to gain access to food and water because this is a an essential need for survival (Matthews 1994). Measurement of the motivation of an animal to obtain a resource will be dependent on the alternatives offered, the elasticity for the demand will be greater when it is more substitutable for a commodity concurrently available (Barnett & Hocking 1981; Lea & Roper 1977). Sherwin & Nicol (1995) combined food searching behaviour with the occurrence of natural obstacles (air stream, water or a narrow gap), thus the willingness to overcome these obstacles to obtain food was used as a yardstick. Dawkins (1981) examined the priorities hens gave to two features of their environment, namely size and flooring of a cage. A comparable approach was used in experiment 2 of the present study, in which commodities addressing related behavioural activities were compared i.e. type of cage flooring (bedding or grid) combined with materials offering shelter (nest box or nesting material). Mice preferred the nesting material although during the night some mice slept in the bedding of the cage with the nest box, but not inside the nest box (see Figure 5). This practical approach allows for the comparison of various environmental aspects and may directly lead to designs for better housing conditions.

Laboratory environments are barren and often poorly structured and contain few features that can be manipulated or changed by the animal's behaviour. This makes it difficult for animals to adopt a behavioural response that reduces the effect of aversive stimuli (coping) in stressful situations (Wechsler 1995). By providing nesting material mice are able to structure their environment by manipulation of the nesting material and this gives them more control over their living conditions. More control may enhance their well-being (Beaver 1989; Chamove 1989a; Van de Weerd & Baumans 1995). Nesting material also allows mice to perform species-specific nest building behaviour. The inability to engage in species-specific behaviour may cause signs of suffering and the mere possibility to perform certain behaviours may decrease the physiological effect of stressful situations (Jensen & Toates 1993). Species-specific behaviour has evolved from continuous adaptations to the natural environment. Despite generations of domestication of mice in the laboratory, adaptive behavioural strategies such as burrowing are still present in laboratory strains and do not appear to be different from wild mice (Adams & Boice 1981). Nest building is related to burrowing activities (Brain & Rajendram 1986) and can be seen as an active strategy of a mouse to control its environment (Sluyter et al 1995).

Housing systems should be designed to allow animals to perform effective coping behaviour when confronted with aversive stimuli, in order to prevent poor welfare (Wechsler 1995). When housing systems cannot be altered immediately, the provision of environmental enrichment such as nesting materials may be a relatively easy, short term solution to enhance well-being. Natural selection, domestication and experience have shaped decision making in animals in such a way that the resultant behaviour is optimally adapted to the current environmental circumstances. In general, this will enhance biological fitness and promote welfare (Fraser 1996; McFarland 1977). It is therefore reasonable to conclude that animal welfare is improved by achieving the objective the animal is willing to work for, and that reaching this objective is experienced as positive (Broom 1988; Van Rooijen 1983/1984). Mice in this study were highly motivated to lie in nesting material, even when presented on a grid floor. Thus we may conclude that nesting material has a positive effect on their well-being.