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Investigations into the preferences of laboratory rats for nest-boxes and nesting materials

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Summary

Nest-boxes and nesting materials were considered potentially useful items with which to enhance the environment of rats housed in standard laboratory cages. This study was carried out to determine whether such items are actually used by rats, and if so, what features are important in their design. Laboratory rats were allowed to choose between four commercially available nest-boxes. Nest-boxes were preferred to other parts of the cage but the nest-box most frequently selected was not suitable for routine laboratory use. Accordingly a new nest-box was designed, incorporating features apparently attractive to the animals. This was a simple structure of opaque perspex, consisting of a roof and three walls. Similarly, rats were exposed to six commercially available nesting materials and those consisting of long paper strips were most preferred.

Keywords Laboratory rats; nest-boxes; shelters; nesting materials; preferences

There are several reasons why the introduction of nest-boxes and nesting material to cages housing laboratory rats might be beneficial to the animals. Firstly, these items would allow rats more control over their ambient temperature. Experimental studies have shown that rats offered a choice of areas with different ambient temperatures select areas of 25°C to 30°C during the light period when they normally rest, whereas during the dark (active) period, they prefer significantly lower temperatures (17°C to 25°C) (Briese 1986, Gordon 1993). Furthermore rats placed in a cold environment (−8°C) will work harder, by pressing a lever in order to obtain heat from an infra-red lamp, during the light than the dark phase, even though this is a time when they would normally be relatively inactive (Carlisle *et al.* 1979).

Secondly, the intensity of lighting in conventional rat housing has been shown to be aversive to albino rats (Blom *et al.* 1995, Schlingmann *et al.* 1994). Providing rats with an opaque or semi-opaque nest-box could therefore increase the comfort of the animals while still providing adequate lighting conditions for caretakers to supervise the animals.

Thirdly, some degree of competitive interaction commonly occurs among laboratory rats housed in groups (Mitchell 1994). Even though this is usually of a benign nature and does not lead to wounding, the provision of an escape area for a rat subjected to aggressive behaviour might be of psychological benefit. This has certainly been the case for group-housed laboratory rabbits, where the introduction of cardboard boxes has provided a refuge for subordinate rabbits (Morton *et al.* 1993).

Fourthly, as the rat is a prey animal, one might expect intuitively that a hiding place in the cage would increase the animal's sense

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of security. Certainly, laboratory rats placed into an outdoor enclosure were found to construct burrows in the earth that were indistinguishable from those made by wild rats (Boice 1977). The provision of a housing system containing 'rudimentary burrows and chambers' has been recommended for laboratory rats (Brain 1992) and a nest-box or nesting material might simulate the relative security of a burrow for rats in laboratory cages.

Fifthly, the fact that the standard laboratory cage provides relatively little stimulation for rats is unarguable. The provision of some source of activity might therefore be of psychological benefit to the animals.

Accordingly, this study was carried out to determine whether rats would make use of either nest-boxes or nesting material introduced to standard laboratory cages, and if so, to define which types were preferred. A literature search revealed that although the preferences of mice for nest-boxes and other shelters have been studied (Buhot-Averseng 1981, Buhot 1987, Ward & DeMille 1991, Van der Weerd *et al.* 1996), similar studies have not been carried out in laboratory rats. Nest-boxes have been commercially produced and tested for mice and some larger versions of these devices were available, but they had not been tested with rats. Some laboratories are using plastic boxes that are produced for storing standard office stationery file cards, to provide shelters in rat cages (C. West, B. J. Howard, personal communications). In this study, we wished to determine whether rats would use any of these commercially available nest-boxes and if so, to determine which were most satisfactory in terms of animal preferences and practicalities. From the results of this first study, we next chose to evaluate two custom-made nest-boxes. Their design was based on important features discovered in our first study.

A literature review was also carried out to determine what nesting materials have been used for laboratory rats. Nesting materials have predominantly been used for peri-parturient female rats and their young; these materials have included wood wool (Norris & Adams 1976), soft-wood shavings and paper (Nolen & Alexander 1966). Pregnant female rats were taught to press a bar in order to obtain nesting material, and bar-pressing increased substantially after parturition (Oley & Slotnick 1970). Although a survey of bedding and nesting materials used in South Africa notes that shredded paper and straw have been provided for laboratory rats, it is not clear what age and sex the animals were (Potgieter & Wilke 1993). The provision of nesting materials for rats is also mentioned in the results of a survey on environmental enrichment in Canadian laboratory animal facilities (Madziak 1992).

The use of nesting materials for rats other than nesting females appears to have declined in recent years, since the third edition of the UFAW handbook (Lane-Petter 1972) notes that 'a small paper tissue is sufficient for rat to make a nest', whereas more recent editions of the same book do not mention the use of such materials at all (e.g. Wiehe 1987). A paper written nearly 70 years ago describes the behaviour of laboratory rats provided with nesting materials; both male and female (non-pregnant and non-lactating) laboratory rats of all ages made nests of paper strips, regardless of prior experience. The size of the nests was temperature dependent, and ceased at ambient temperatures of 27°C and higher (Kinder 1927). Another paper indicating the importance of nest-building in the behavioural repertoire of rats is that of Boice (1977). He noted that laboratory rats placed in outdoor earth enclosures and provided with paper nesting material made burrows in the earth and dragged the paper into underground nesting chambers. Scharmann (1991) also observed that when pieces of cellulose paper or straw were placed on cage tops, laboratory rats would pull these through into the cage and 'some rats used straw for furnishing their nesting place'. Irradiated straw is also used successfully as a nesting material for laboratory rats in one British animal facility (S. Cubitt, personal communication). However, unless straw of genuine organic quality is obtained, its chemical composition cannot be guaranteed and it may not therefore be suitable for animals used in toxicological testing.

Important considerations for selecting any nest-box or nesting material were that they should be (i) suitable for use in toxicological studies, being preferably inedible or if edible, of known chemical composition, (ii) sterilizable or free from harmful microorganisms, and (iii), economical to purchase or produce and therefore suitable for implementation on a large scale.

Materials and methods

Animals and housing

Thirty-six male Sprague-Dawley rats (virus antibody free CR1:CD BR rats; Charles River UK Ltd, Margate, Kent), aged 3 to 4 weeks and weighing approximately 100 g, were purchased and randomly distributed into groups of three, a group size recommended by Mitchell (1994). Three of these groups were randomly assigned to pilot studies. Each group was housed in a polypropylene cage (RC2 cage; North Kent Plastic Cages Ltd, Erith, Kent) measuring 56 × 38 × 20 cm, supplied with sawdust bedding (Lignocel BK8/15; RS Biotech, Finedon, Northants). The animals were kept under barrier conditions, with a minimum of 20 air changes per minute. Feeding was *ad libitum* with a complete expanded diet (R&M1 SQC(E); Special Diets Services Ltd, Witham, Essex) and mains water was available, also *ad libitum*, from water bottles. Temperature and humidity were fully controlled at 21°C ± 2 and 55% ± 10. The light:dark period was 9 h:15 h, with lights on at 08:00 h and off at 17:00 h. Lighting was provided by three desk lamps, fitted with 60 w bulbs and arranged so as to provide a light intensity of 120 lux in the centre of the room and 45 to 65 lux in the area of the room in which preference testing was to be carried out. This relatively low light intensity was used to facilitate exploration by rats in preference cages, where a conventional light intensity appears to be aversive (Manser *et al.* 1995). During the dark period, a dim red light (7 lux at 'rat level') was used to provide sufficient illumination for video recording.

The rats were allowed to acclimatize for a period of 2 weeks. Meanwhile, they were handled during the weekly cleaning out operation, and in addition, by the experimenter twice a week during the first 4 weeks and once a week thereafter. On these occasions, each rat was briefly stroked over the shoulders, then picked up around the shoulders and thorax for a few seconds before being replaced on the floor of the cage. This procedure has previously been found to be effective in facilitating the handling of rats in preference cages (Manser *et al.* 1995).

Nest-boxes

The following items were purchased:

NB1: 'hanger' (International Market Supply, Congleton, Cheshire) in clear perspex (Perspex™ or Plexiglass). With solid sides and roof; open at both ends and to the floor. Size 20 cm × 12.5 cm × 12 cm high

NB2: 'hanger' (International Market Supply, Congleton, Cheshire) in smoky (semi-opaque) perspex

NB3: an empty box (WH Smith & Son, Ltd, Retail newsagents and booksellers) of solid plastic (20.5 cm × 12.5 cm × 17 cm high) as used for standard office stationery file cards. A hole, 9 cm high and 10 cm wide, was cut into the front of the box, to allow entry

NB4: vertical partition 20 cm × 25 cm high, made in smoky perspex, fixed approximately 12 cm away from the side wall of the cage

These nest-boxes are shown in Figs 1 to 4 inclusive.

Following initial pilot and preference studies, perspex nest-boxes with the following dimensions were produced: 25 cm (length), 17 cm (depth) and 12 cm (height). These were open to the floor and open to the front. Two prototypes were made, the first box (NB5) being of semi-opaque, smoky brown perspex and the second (NB6) of opaque black perspex (Fig 5).

Nesting materials

Six commercially available nesting materials were identified as suitable for pilot investigations, being non-toxic, non-traumatic and low in pathogens. These were:

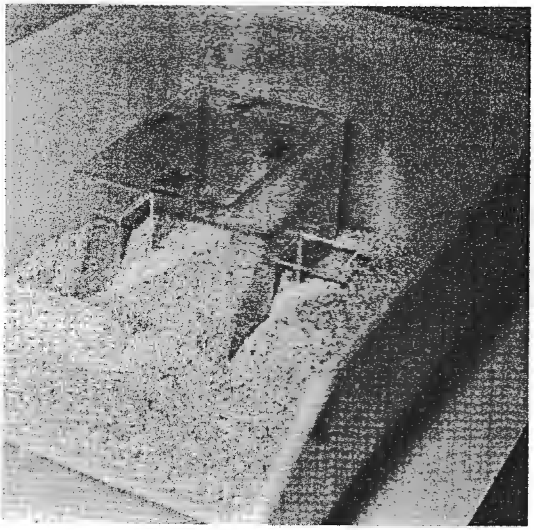

Fig 1 'Hanger' in clear perspex (NB1)

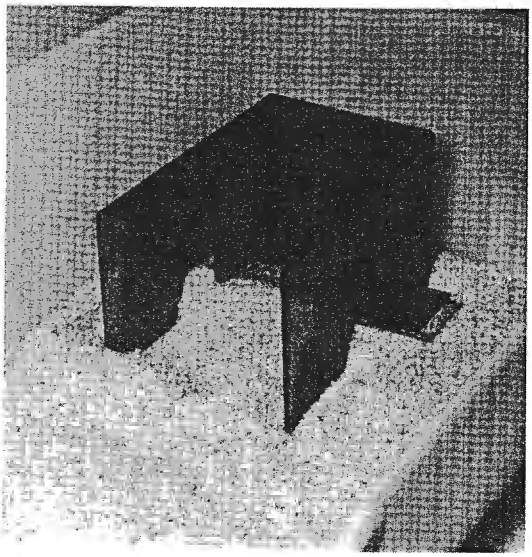

Fig 2 'Hanger' in smoky (semi-opaque) perspex (NB2)

NM1: soft paper shavings (RS Biotech, Finedon, Northants), approximately 1–2 cm long and 3 mm wide
NM2: soft paper strips (5 mm supersoft nesting material; RS Biotech, Finedon, Northants), approximately 40 cm long and 5 mm wide
NM3: soft paper strips (10 mm coarse nesting material; RS Biotech, Finedon, Northants), approximately 40 cm long and 10 mm wide

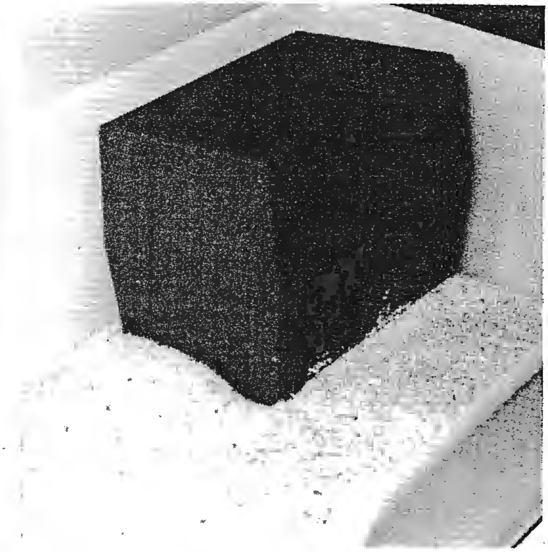

Fig 3 Empty solid plastic box (NB3)

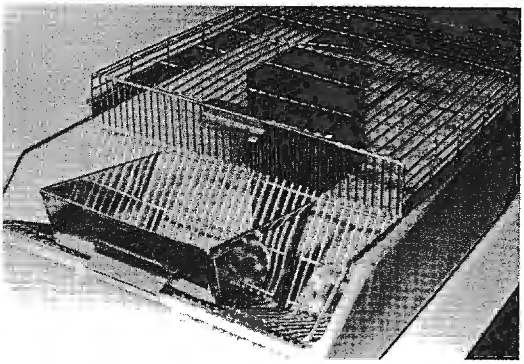

Fig 4 Cage with smoky perspex vertical partition (NB4)

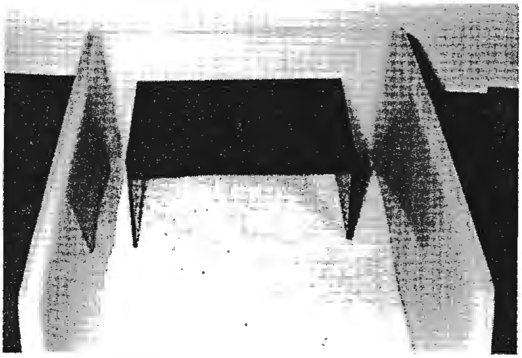

Fig 5 Prototype box of opaque black perspex (NB6), open to the floor and front

NM4: coarse paper strips ('Enviro-dri'; Wm. Lillico & Son, Betchworth, Surrey), approximately 12 cm long and 3 mm wide

NM5: wood shavings (Goldshavings; Wm. Lillico & Son, Betchworth, Surrey), approximately 1–2 cm long and 0.5–1 cm wide

NM6: 5 cm squares of compressed cotton fibre ('Nestlets', ANcare Corp. Bellmore, NY, USA).

Test apparatus

An area of the animal room was set aside for observational and preference studies. Up to four standard cages or two preference testing systems could be placed in the test area. The preference testing system consisted of two cages, identical to those in which the rats were housed, joined to a central box by means of clear perspex tubes, 6 cm long and 8.5 cm in internal diameter. Food and water were available in both test cages. The central box, also made of clear perspex, measured 30 cm × 15 cm × 19.5 cm high and contained a wire grid floor, in order to make it less attractive to the rats than the two cages. Preference systems were placed on the floor in parallel, with wooden boards between them to reduce visual, and possibly auditory, contact between the test rats. The light intensity measured in the centre of each nest-box, when placed in the test area, is shown in Table 1.

A video camera, connected to a time-lapse video recorder and monitor, was suspended over the test area using a Panasonic CCTV video camera (Hitachi VT-L2000E time-lapse video cassette recorder and Sony Trinitron colour TV monitor). The recorder and monitor were placed at a distance of approximately 1.2 m from the test area in order to reduce ultrasonic sound which can be disturbing to laboratory rodents (Sales *et al.* 1989).

Table 1 Light intensities measured in the test room and in the centre of each nest-box, when placed in the test area

Area	Light intensity (lux)
Centre of room	120
Test area	45–65
Nest-box NB1	45
Nest-box NB2	32
Nest-box NB3	6
Nest-box NB4	45
Nest-box NB5	32
Nest-box NB6	8

Experimental methods

Experiment 1: Assessment of commercially available nest-boxes

Pilot studies were conducted to determine firstly, whether rats were likely to use the selected nest-boxes, and secondly, how much time was required for habituation before they would occupy the boxes. Observations of two individual rats were carried out for each nest-box. Each rat was placed into a cage identical with its home-cage and containing one of the nest-boxes, one hour before the onset of the dark period. The rat remained in this cage for 72 h, during which time its behaviour was recorded by means of time-lapse video recording.

Preference tests were conducted to determine which of the nest-boxes was used most frequently, by placing a different nest-box in each of the two cages in the preference system. A balanced incomplete block design was used to allocate nest-boxes to the cages, such that each possible pairing of nest-boxes was tested. There were six possible combinations of two nest-boxes. Three replicates of the design were tested, using a total of 18 rats.

The rats were randomly allocated for use in these tests and were placed individually into the centre box of the test system, then allowed 24 h to acclimatize before video-recording was carried out over the next 24-h period. The position of the rat, whether in contact with a nest-box or elsewhere in the preference system, was recorded every 10 min during this 24-h period. The position was only recorded as 'in contact with a nest-box' if the rat was inside, on top of, or lying with part of the body against the nest-box. The occupancy of each nest-box as a percentage of total time was calculated. Since there was a negative correlation between the times spent in each cage, each of the six pairings of nest-boxes was regarded as a 'treatment'. The response to a treatment was calculated as the difference in percentage time spent in each box, that is:

$$\% \text{ difference} = \% \text{ time in NB}_i - \% \text{ time in NB}_j$$

where box number suffix $i < j$

The treatments were as follows:

1 = Time spent in NB1 when NB2 also available
2 = Time spent in NB1 when NB3 also available
3 = Time spent in NB1 when NB4 also available
4 = Time spent in NB2 when NB3 also available
5 = Time spent in NB2 when NB4 also available
6 = Time spent in NB3 when NB4 also available

The data were analysed in the SAS package using a two-way analysis of variance for a randomized complete block design in the form:

$$\begin{aligned}\% \text{ difference} = {} & \text{Intercept} + \text{Replicate}_i \\ & + \text{Dark}_j + \text{Treatment}_k \\ & + \text{Dark}_j{*}\text{Treatment}_k + \text{Error}\end{aligned}$$

where Intercept is the overall response mean. Replicate adjusts for any differences between the three replicates of the design. Dark tests the effect of light and dark periods. Treatment is as defined above. Dark*Treatment interaction tests whether the treatment effects are different in dark and light periods.

Experiment 2: Assessment of two nest-boxes of novel design

Following the results of these experiments, testing was carried out on the two newly designed nest-boxes. Pilot observations were carried out in which two rats were each housed singly in a cage containing NB5 and two housed in a similar way with NB6. Two nest-boxes of each type were also added to cages housing three rats (one nest-box to each of four cages). All of the rats were observed by time-lapse video recording over a 48-h period.

Six naive rats were tested individually in a preference system as described in Experiment 1, with NB5 in one cage and NB6 in the other. The entire test period of 24 h was recorded by time-lapse video recording and on reviewing the videotape, the position of each rat was noted at 10-min intervals. As in Experiment 1, the percentage of time spent by each rat in one of the nest-boxes or elsewhere in the test system was calculated. The difference in mean percentage occupancy time between NB5 and NB6 was calculated separately for the light and dark periods, along with 95% confidence intervals for the differences.

Experiment 3: Assessment of commercially available nesting materials

Two pilot trials, using individual rats, were carried out for each nesting material. Each rat was placed into a cage identical to its home-cage and containing one of the nesting materials, at approximately 16:00 h. A plastic measuring jug was used to measure and deliver 2000 cm^3 of nesting materials NM1 to NM5 into each test cage; alternatively, 12 compressed cotton squares (NM6) were added to a test cage. The rat remained in the test cage for 72 h, during which time its behaviour was recorded by means of time-lapse video recording, as previously described. A further two pilot trials were also conducted for each nesting material in a similar way, but using groups of three rats.

Having determined which nesting materials were used by rats in the pilot studies, choice tests were carried out in which a different material was placed into each of the cages in the preference testing system described in Experiment 1. Rats were tested individually, starting each test at 16:00 h and recording their position in the test system over 24 h; no prior exposure was considered necessary. Since the first four rats tested all persisted in carrying the nesting materials from one cage to another and mixing them up, this methodology was abandoned. Large quantities of nesting material (up to 4000 cm^3 per cage) were tried in case the rats simply wished to make larger nests, but mixing of materials still occurred.

The method used, therefore, was to place 3000 cm^3 of each nesting material into one cage of the preference system, and to leave the other cage empty. The rat being tested thus had the choice of being in contact with the nesting material or out of contact, even if it did carry the material around the test system. Six rats were exposed to each of the nesting materials found suitable in the pilot studies. Again, no prior exposure was found necessary, so each rat was placed into the test system for 24 h and observed by means of time-lapse video recording throughout the period. The mean percentage time spent with each of the three nesting materials in both dark and light periods was calculated. An analysis of variance was carried out to determine whether there was an effect of light condition, material used or any interaction between light condition and material.

Results

Experiment 1: Pilot studies

The rats in this study had the option of dwelling in a nest-box or elsewhere in the cage. An examination of the percentage of time the rats spent either in, on top of or in contact with each nest-box, shows that they frequently chose to occupy a nest-box. Rats were often observed climbing onto NB1 or NB2 during active periods. Rest periods of up to 3 h were also taken during the dark period, frequently in or on the nest-boxes. During the light period, the rats rested all the time, although a short foray to feed was sometimes observed, usually between 12:00 and 13:30 h. A resting site was normally established early in the light period, and the rat would remain there until darkness, although a few rats moved to a new resting site during the day. All of the nest-boxes were used for resting throughout most of the light period, although the transparent NB1 was less popular than the other nest-boxes at this time (Fig 6). When rats rested elsewhere in the cage, a cage corner was the most likely site to be chosen.

Occupancy rates of nest-boxes NB1, 2 and 4 were similar during the first, second and third 24-h period but the rats appeared to be

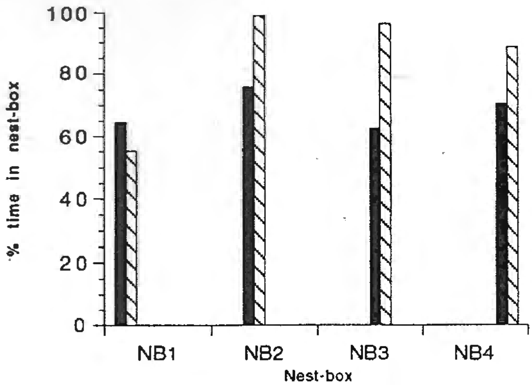

Fig 6 Percentage times rats observed in nest-boxes during instantaneous scans every 10 min in the second 24-h period of the pilot study. Dark blocks represent time during dark period; light blocks represent time during light period

reluctant to enter NB3, thus spending less time in it during the first 24 h than subsequently. Occupancy rates for this box were similar during the second and third 24-h period. All of the nest-boxes were considered to merit further investigation by means of preference testing.

Experiment 1: Preference studies

Since the rats had shown some reluctance to enter NB3 during the first 24 h, a period of 24-h acclimatization to the nest-boxes was allowed before the start of preference testing. Even after a 24-h period of habituation, the rats used in preference testing actively explored the test system during the dark period, running through the perspex tubes and centre box, from one cage to the other. There was no evidence that the rats were aware of rats in other cages and movements of rats in adjacent test systems were not synchronized.

Analysis showed that there was a significant difference between treatments ($F_{5,22}=24.09$, $P=0.0001$), with nest-box NB3 being chosen most frequently, followed by nest-box NB2 and finally NB1 and NB4 (Fig 7). There was also a significant difference ($F_{5,22}=5.12$, $P=0.0029$) between preferences during the dark and the light period. Rats which were exposed to nest-box NB3 were highly likely to choose this for resting, particularly if it was paired with nest-boxes NB1

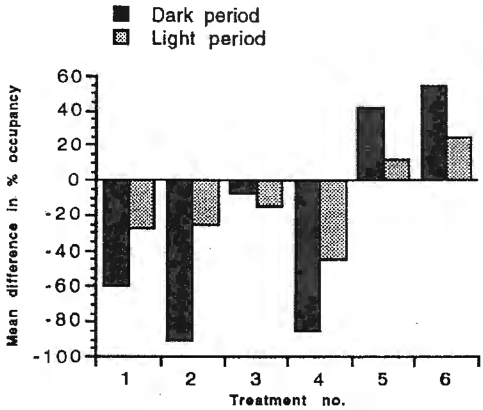

Fig 7 Mean preferences in each treatment group in Experiment 1 (mean = 0% if there was no preference). Treatments were as follows: 1 = 'NB1 given NB2'; 2 = 'NB1 given NB3'; 3 = 'NB1 given NB4'; 4 = 'NB2 given NB3'; 5 = 'NB2 given NB4'; 6 = 'NB3 given NB4'

Table 2 Mean percentage times each nest-box was chosen during preference trials in Experiment 1

	NB1	NB2	NB3	NB4
Times chosen during dark period	9.6	21.5	39.9	10.2
Times chosen during light period	2.4	29.3	78.7	4.2

or NB4, which were rarely chosen. The mean percentage of times each nest-box was chosen in the preference trials is shown in Table 2.

Considerations of nest-box design

Although nest-box NB3 was most frequently chosen by the rats, it was not considered to be a suitable nest-box for practical purposes. The lid could not be raised when it was *in situ*, so that rats inside the box could only be inspected after removing the cage from the rack. The nest-box was also too small for a group of three pilot rats to rest in, even when the whole of the lower front of the box had been removed. Furthermore, it did not appear sufficiently durable for long-term use. Hence, a decision was taken to design new nest-boxes (NB5 and NB6).

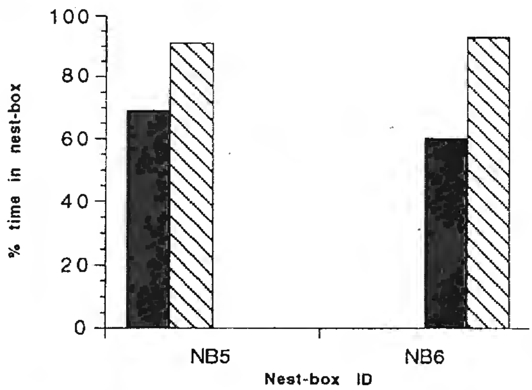

Fig 8 Percentage times spent in nest-boxes NB5 and NB6, by individual rats in pilot studies. Dark blocks represent time during dark period; light blocks represent time during light period

Experiment 2: Pilot studies

Rats housed singly in a cage with either NB5 or NB6 showed no reluctance to enter either of the nest-boxes. They spent a large proportion of the dark period on top of the nest-box, either actively investigating the environment or resting. All rats chose to rest in a nest-box for most of the duration of the light period. Mean occupancy rates of NB5 and NB6 respectively were 69.0% and 60.4% during the dark period and 91.0% and 93.2% during the light period (Fig 8).

The groups of rats housed with the nest-boxes also spent a great deal of time during the dark period on top of the nest-boxes. Usually only one or two rats would occupy this position. At the beginning of the light period, all three rats were usually seen to enter the nest-box and remain there. The nest-boxes appeared sufficiently large for all of the rats, which now weighed between 570 g and 700 g. The mean occupancy rate of NB5 by groups of rats was 69.6% during the dark period and 87.6% during the light period, whereas equivalent figures for NB6 were 89.8% and 97.8% respectively (Fig 9).

Experiment 2: Preference studies

Rats tested in the preference systems spent a great deal of active time exploring both cages and climbing on to the nest-boxes. Comparison of the times spent in NB5, NB6 or elsewhere in the test system during the dark

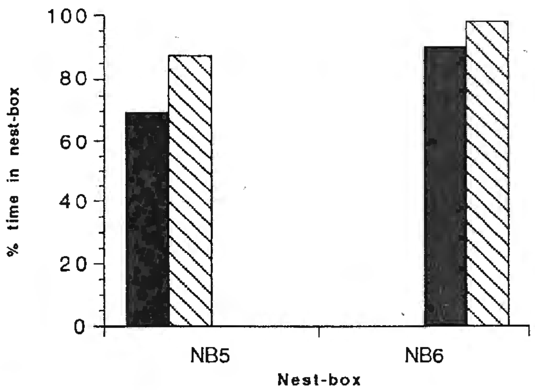

Fig 9 Mean percentage times spent in nest-boxes NB5 and NB6, by groups of three rats in pilot studies. Dark blocks represent time during dark period; light blocks represent time during light period

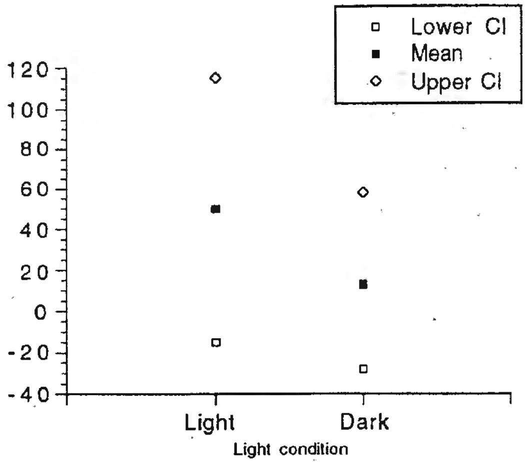

Fig 10 95% confidence limits for the difference in preference for NB5 and NB6 during the light and dark periods. (Preference = NB6%–NB5%, so a positive number indicates a preference for NB6)

Table 3 Mean percentage times each nest-box was chosen during preference trials in which nest-boxes NB5 and NB6 were offered

	NB5	NB6
Times chosen during dark period	25.4	39.0
Times chosen during light period	15.2	64.8

period showed no significant difference between the occupancy rates of the two nest-boxes. Although the rats appeared to spend more time in NB6 than NB5 during the light period (Table 3), the difference did not quite reach statistical significance. As shown in Fig 10, the 95% confidence intervals for the differences between occupancy of NB5 and NB6 in the dark and light period are relatively large and both contain the value zero.

Experiment 3: Pilot studies

The rats showed no hesitation in approaching any of the nesting materials tested, but both individual and group-housed animals scattered nesting materials NM1 and NM5 around the cage, mixed them up with the sawdust bedding and then ignored them. NM6 was torn into shreds and also scattered around the cage and ignored. However all of the pilot rats manipulated the remaining three nesting materials (NM2, NM3 and NM4) and rested on them both during the light period and during short rests taken during the dark period. The rats tended to make shallow depressions in the nesting material rather than build nests.

Experiment 3: Preference studies

Rats which participated in preference tests spent considerable periods of time in contact with all of the three nesting materials. During the dark periods, the rats moved around in the materials or manipulated them. During the light period, the rats would rest in a corner of the cage, surrounded by the material. The soft paper nesting materials were used more extensively because some rats carried pieces around and placed them in the centre box as well as in both cages of the test system. Thus the rats tested with these materials were more likely to be in contact with them. They also sometimes chose to rest in the centre box on a mat of paper strips. Conversely, although rats often rested on NM4, this was rarely moved out of the cage into which it was first placed.

The analysis of variance was used to predict treatment means ('least square means') for light and dark periods from the fitted model. The predicted treatment means were

Table 4 Mean percentage times spent with each nesting material in preference trials

	NM2	NM3	NM4
Light period	81.4	98.0	66.3
Dark period	73.1	81.4	51.8

plotted. The ranking of the means was not tested statistically, but the following conclusions were made by interpreting the plot. The percentages of time spent in contact with each of the nesting materials were significantly different ($P = 0.0111$), and ranking of these preferences was in the order of NM3 > NM2 > NM4 (Table 4). The differences between preferences during the dark and light periods were not as marked as was seen in the nest-box studies ($P = 0.1404$).

Discussion

Nest-boxes

The principal results of these studies was that rats make use of a nest-box, especially if it provides protection from light. The results show that occupancy times for nest-boxes NB1, 2, 3 and 4 were very much higher in the pilot studies than in the preference trials. However, this can easily be explained because the rats spent time in both nest-boxes in the preference trials, thus lowering the time they could occupy one nest-box. Also, the rats spent a great deal of time in patrolling the relatively large and complex space provided by the preference test system. The differences in occupancy depending upon the nest-boxes available were marked, so that rats offered NB2 or NB3 were likely to spend long periods in the nest-boxes, whereas rats offered a choice between NB1 or NB4 spent more time in the open than in one of these nest-boxes. Presumably these did not offer much security to the rats which were in a relatively large and novel environment.

The reasons why rats were more likely to occupy NB3 than other nest-boxes were considered. The opaque walls may have been attractive since they would provide a refuge from the light. The lower light intensity inside NB2 may explain why it was more popular than NB1. Interestingly, the rats appeared to prefer the darker nest-boxes even during the dark period; possibly the environment did not appear completely dark to the rats, although they showed intense activity during this time. A more likely possibility is that since the rats had experience of the nest-boxes during the light period, they had already determined that the darker boxes were more secure. It is interesting to note that in a study of nest-box preferences in mice, nest-boxes with perforated walls were preferred to those with solid ones (Van der Weerd *et al.* 1996).

Another feature which may have rendered NB3 attractive to the animals is its enclosed corners, which were also provided to some extent by NB4 but not at all by NB1 and NB2. However the ability of the rats to climb on top of NB1 and NB2 meant that these boxes not only increased available space for the rats, but they also provided them with a source of activity. Although rats in pilot studies made use of the vertical partition NB4 by resting behind it, the partition was not preferred to any of the nest-boxes. Cage partitions may be of some value to laboratory rodents, however, since a cage with partial vertical dividers was preferred by laboratory rats to an unenhanced cage or one divided horizontally (Anzaldo *et al.* 1994). Furthermore, laboratory mice housed in cages containing vertical partitions appeared less stressed than those in an open cage (Chamove 1989).

Nest-box NB3 was not considered suitable for day-to-day use because its design made inspection of the rats difficult, it was too small for three fully grown rats and it was not sufficiently durable. Hence, a decision was made to design a novel nest-box in smoky (NB5) or opaque (NB6) perspex, which incorporated the features apparently favoured by the rats in the study of commercially available nest-boxes. The results of the pilot observations showed that both NB5 and NB6 were readily accepted and used in preference to other parts of the cage. The numbers of rats used in the pilot studies were too small to allow for statistical testing but there appeared to be little difference between durations of occupancy for each of the two nest-boxes. The rats used in preference tests were also more likely to occupy a nest-box than to be elsewhere in the test system. Although mean occupancy rates were higher for NB6 than NB5, the differences did not reach statistical significance. The fact that NB5 appeared to be less favoured during the light period than NB6 was probably because it would have offered less protection from the light. The lack of significance in the results indicates that either nest-box would be appropriate for use in laboratory cages. The possible preference shown by the rats for NB6 might be outweighed by the advantage of easier monitoring of rats in NB5.

Nesting materials

In these studies, the three nesting materials which consisted of long paper strips were found to be attractive to the rats. They showed no hesitation in approaching these materials and spent a great deal of time in manipulating them. However, elaborate nests, as sometimes seen in mouse cages (Sherwin 1997), were not observed. This may have been partly due to a behavioural difference between rats and mice and partly related to the thermoneutral ambient temperature provided, since Kinder (1927) noted that the size of nests built by laboratory rats was proportional to the surrounding air temperature. The rats appeared to be unwilling or unable to create a nest from small pieces of paper, cotton or wood material, and these were simply scattered around the cage.

This preference for nesting material of relatively large particle size is in accordance with the finding of Blom *et al.* (1996), that both rats and mice prefer coarse to fine bedding materials. The mice in their study also much preferred shredded paper to sawdust or woodchip bedding. Van der Weerd *et al.* (1997) considered that mice selected nesting materials primarily on the basis of their 'nestability', or effectiveness in nest-building. These findings indicate that there should possibly be a change from conventional rodent bedding and nesting materials. However, it is important to be aware that some wood-derived materials can be hepatotoxic and enzyme-inducing in rodents (Potgieter *et al.* 1995).

Combining nest-boxes and nesting materials

Informal, longer-term studies showed that paper nesting materials could be successfully used in conjunction with nest-boxes. The rats would line the nestboxes with the paper strips, which they collected from the hopper area of the cage lid. Behavioural observations showed that there were no problems with aggression between rats provided with either nest-boxes, nesting materials or both. Three rats would frequently occupy the top of the nest-box during active periods, and would then enter the box for rest periods.

Conclusions

The study of commercially available nest-boxes provided information about rats' preferences, which was useful in the design of a purpose-built nest-box. Nest-boxes of opaque or semi-opaque materials were preferred to transparent ones and those with enclosed corners were preferred to nest-boxes with open ends. A height of approximately 12 cm allowed rats to climb on top of the box, and considerable periods were spent in this situation, particularly during the dark period. The nest-box designed in this study could be easily removed from the cage and when the box was *in situ*, animals could be easily monitored or caught. Perspex appeared to be a suitable material for nest-boxes since it is easy to clean and was not chewed by rats, even when the nest-boxes were kept in cages over an 8-week period. The nest-boxes designed in this study therefore fulfilled the criteria of acceptability to the rats and practicality; they can also be produced at a low cost. While this study has served to demonstrate that rats are attracted to such nest-boxes, there may be other designs and materials which would be equally appropriate.

The rats in this study appeared to be very attracted to paper nesting materials, and spent almost 100% of the light period resting on the most favoured material. The materials were also extensively used during the dark period, when their manipulation provided the rats with a considerable amount of activity. None of the materials appeared to be ingested and the favoured paper material was a commercially available laboratory animal bedding of good quality and therefore likely to be low in pathogens and carcinogens. Sterilization of the materials by exposure to ultra-violet light would further enhance their safety to the animals.

Whereas nest-boxes were used for longer periods during the light period than the dark, contact with the nesting materials was similar during both periods. The reason for this is probably that the rats used the materials for resting as well as for activity, such as burrowing and manipulation.

This study has shown that both perspex nest-boxes and paper nesting materials are used extensively by laboratory rats. A further paper will describe how the strength of preference for such items was investigated. Both items require little extra in terms of labour or financial outlay, and can be suitable for animals used in toxicological studies. The provision of nest-boxes and nesting materials therefore appear to be an effective and practical method of improving the cage environment of laboratory rats.

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References

Anzaldo AJ, Harrison PC, Riskowski GL, *et al.* (1994) Increasing welfare of laboratory rats with the help of spatially enhanced cages. *Animal Welfare Information Center Newsletter* **5**(3), 1,2,5

Blom HJM, Van Tintelen G, Baumans V, Van Den Broek J, Beynen AC (1995) Development and application of a preference test system to evaluate housing conditions for laboratory rats. *Applied Animal Behaviour Science* **43**, 279–90

Blom HJM, Van Tintelen G, Van Vorstenbosch CJAHV, Baumans V, Beynen AC (1996) Preferences of mice and rats for types of bedding materials. *Laboratory Animals* **30**, 234–44

Boice R (1977) Burrows of wild and albino rats: effects of domestication, outdoor raising, age, experience and maternal state. *Journal of Comparative Physiology and Psychology* **91**, 649–61

Brain PF (1992) Understanding the behaviours of feral species may facilitate design of optimal living conditions for common laboratory rodents. *Animal Technology* **43**, 99–105

Buhot M-C (1987) Mouse exploration and choice of nestboxes differing in size. *Animal Learning and Behavior* **15**, 382–94

Buhot-Averseng M-C (1981) Nest-box choice in the laboratory mouse: preference for nest-boxes differing in design (size and/or shape) and composition. *Behavioural Processes* **6**, 337–84

Briese E (1986) Circadian body temperature rhythm and behavior of rats in thermoclines. *Physiology and Behavior* **37**, 839–47

Carlisle HJ, Wilkinson CW, Laudenslager ML, Keith LD (1979) Diurnal variation of heat intake in ovariectomised, steroid-treated rats. *Hormones and Behavior* **12**, 232–42

Chamove AS (1989) Cage design reduces emotionality in mice. *Laboratory Animals* **23**, 215–19

Gordon CJ (1993) Twenty-four hour rhythms of selected ambient temperature in rat and hamster. *Physiology and Behavior* **53**, 257–63

Kinder EF (1927) A study of the nest-building activity of the albino rat. *Journal of Experimental Zoology* **47**, 117–61

Lane-Petter W (1972) The laboratory rat. In: *The UFAW Handbook on the Care and Management of Laboratory Animals*, 4th edn. Edinburgh and London: Churchill Livingstone, pp 204–11

Madziak RS (1992) Environmental enrichment from a management perspective. In: *Workshop Proceedings. Animal Care Committees: Roles and Responsibilities*. London, Ontario, Canada: Canadian Association for Laboratory Animal Science, pp 96–108

Manser CE, Morris TH, Broom DM (1995) An investigation into the effects of solid or grid cage flooring on the welfare of laboratory rats. *Laboratory Animals* **29**, 353–63

Mitchell PJ (1994) Ethological studies of the social behaviour of the rat. *Animal Technology* **44**, 109–20

Moreton DJ (chairman) *et al.* (1993) Refinements in rabbit husbandry. Second report of the BVAAWF/FRAME/RSPCA/UFAW joint working group on refinement. *Laboratory Animals* **27**, 301–29

Nolen GA, Alexander JC (1966) Effects of diet and types of nesting material on the reproduction and lactation of the rat. *Laboratory Animal Care* **16**, 327–36

Norris ML, Adams CE (1976) Incidence of pup mortality in the rat with particular reference to nesting material, maternal age and parity. *Laboratory Animals* **10**, 165–9

Oley NN, Slotnick BM (1970) Nesting material as a reinforcement for operant behavior in the rat. *Psychonomic Science* **21**, 41–3

Potgieter FJ, Törrönen R, Wilke PI (1995) The *in vitro* enzyme-inducing and cytotoxic properties of South African laboratory animal contact bedding and nesting materials. *Laboratory Animals* **29**, 163–71

Potgieter FJ, Wilke PI (1993) Laboratory animal bedding and nesting materials for rodents and lagomorphs: the South African situation. *Journal of the South African Veterinary Association* **64**, 144–8

Sales G, Evans J, Milligan S, Langridge A (1989) Effects of environmental ultrasound on behaviour of laboratory rats. In: *Laboratory Animals Welfare Research-Rodents*, Potters Bar, Herts: UFAW, pp 7–15

Scharmann W (1991) Improved housing of mice, rats and guinea-pigs: a contribution to the refinement of animal experiments. *Alternatives to Laboratory Animals* **19**, 108–14

Schlingmann F, de Rijk SHLM, Pereboom WJ, Remie R (1994) 'Avoidance' as a behavioural parameter in the determination of distress among albino and pigmented rats at various light intensities. *Animal Technology* **44**, 87–96

Sherwin CM (1997) Observations on the prevalence of nest-building in non-breeding TO strain mice and their use of two nesting materials. *Laboratory Animals* **31**, 125–32

Van der Weerd HA, van Loo PLP, van Zutphen LFM, Koolhaas JM, Baumans V (1996) Preferences for nest boxes as environmental enrichment for laboratory mice. In: *Environmental Enrichment for Laboratory Mice: Preferences and Consequences*, Chap 4 (PhD thesis). University of Utrecht

Van der Weerd HA, van Loo PLP, van Zutphen LFM, Koolhaas JM, Baumans V (1997) Preferences for nesting material as environmental enrichment for laboratory mice. *Laboratory Animals* **31**, 133–43

Wiehe WH (1987) The laboratory rat. In: *The UFAW Handbook on the Care and Management of Laboratory Animals*, 6th edn. (Poole T, ed). London: Longman Scientific & Technical, pp 309–30