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Operant studies to determine the strength of preference in laboratory rats for nest-boxes and nesting materials

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Summary

Previous work has shown that laboratory rats preferred to use nest-boxes and nesting materials rather than empty parts of the cage. In preference tests, they chose opaque or semi-opaque nest-boxes and long strips of soft paper nesting material. Choice tests to demonstrate a preference between nest-boxes and nesting material were not possible because nesting materials were carried into the nest-boxes. Furthermore, preference tests did not show how important these items were to the animals. Accordingly, operant tests were conducted, in which the rats had to lift a weighted door in order to gain access to an empty cage, or one containing a nest-box, nesting material or both items. By progressively increasing the weight of the door in subsequent trials, it was shown that the rats would carry out more work to reach a nest-box, with or without nesting material, than to reach an empty cage.

Keywords Laboratory rats; nest-boxes; shelters; nesting material; preferences; strength of preference; operant studies

Previous work has shown that laboratory rats made use of both nest-boxes and nesting materials (Manser *et al.* 1998). They preferred an opaque or semi-opaque nest-box to other parts of the cage, and they preferred a cage furnished with paper nesting material to one without. Choice studies were used to test different plastic and perspex nest-boxes, as well as paper and wood nesting materials. Consideration was also made of whether the items were economical, hygienic, suitable for use in toxicological studies and allowed routine monitoring of the rats. The most suitable nest-box found was made of opaque perspex, with solid sides and roof and open front and base. Its dimensions were: 25 cm (length), 17 cm (depth) and 12 cm (height). The preferred nesting material, which also fulfilled the above criteria, consisted of soft paper strips, approximately 10 mm wide and 40 cm long (10 mm coarse nesting material; RS Biotech, Finedon, Northants). However, since the rats tended to carry nesting material around, it had not been possible to devise a test system which would show whether the rats preferred nest-boxes or nesting materials. Also, the importance of such commodities to the rats was not previously demonstrated. The usefulness of preference studies is limited by the fact that they merely demonstrate that one commodity may be preferred to another and they do not show how important it is to the animal to have made that choice (Duncan 1992). Therefore, in order to answer both these questions, operant studies were used to determine whether rats would work to obtain these commodities and if so,

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whether they would put more effort into reaching one than the other.

The method used to examine strengths of preference in this study was based on that described by Manser *et al.* (1996), in which rats are required to lift progressively increasing weights in order to reach a favoured commodity. The purpose of this experiment was to compare the weight which a rat would to lift in order to reach (i) an empty cage, (ii) a cage containing a nest-box, (iii) a cage containing nesting material or (iv) a cage containing both nest-box and nesting material.

Materials and methods

Animals and housing

Eight male Sprague-Dawley rats (virus antibody free CR1: CD BR rats; Charles River UK Ltd, Margate, Kent) were used, all of which had experience of the preference system as described by Manser *et al.* (1998) but which had no experience of either the nest-box used in this study, or of any nesting material. The rats were now aged 7 months and weighed between 700 g and 840 g.

The rats were housed with others not used in the experiment, in groups of three, in polypropylene cages measuring 56 × 38 × 20 cm (RC2 cage; North Kent Lastic Cates Ltd, Erith, Kent), 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 hour. Feeding was *ad libitum* with a complete expanded diet (R&M1 SQC(E); Special Diets Services Ltd, Witham, Essex) and mains water was also *ad libitum*, from water bottles. 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 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 and operant testing (Manser *et al.* 1995).

Test apparatus

The test system consisted of two cages, identical to those in which the rats were housed, joined to a central box by means of perspex tubes, 6 cm long and 8.5 cm in internal diameter. The central box, also made of 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. A central barrier was inserted into the centre box during the trials. This barrier could be adjusted to any height by means of a screw on the outside of the box. Its weight was approximately 110 g and this could be increased to 460 g by adding weights to it (Fig 1). A spring-loaded pawl on the side of the centre box, which engaged with a ratchet on the barrier prevented the latter from dropping again once the rat had lifted it (Fig 2). Two test systems were used at a time and they were placed on the floor of

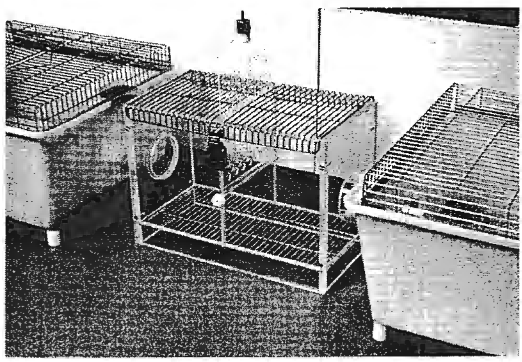

Fig 1 Test system of two cages with central box

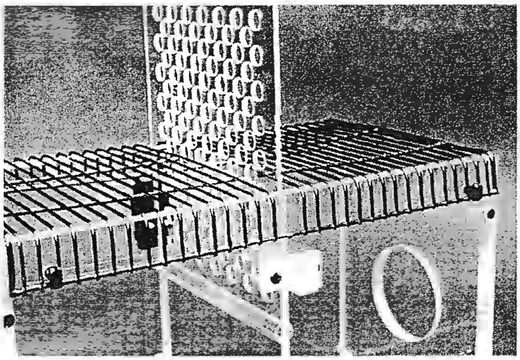

Fig 2 Barrier in central box of test system

the animal room in which the rats were housed. The systems were separated from each other and from the rest of the room by solid wooden boards 30 cm high, in order to minimize visual, auditory and olfactory contact.

Operant testing

A 2 × 2 factorial design was used, with each rat being offered a pair of cages joined together. Four different pairs of cages were used:

(i) two empty cages
(ii) an empty cage and a cage containing a nest-box
(iii) an empty cage and a cage containing nesting material
(iv) an empty cage and a cage containing both nest-box and nesting material.

Four rats were used in a crossover design, so that each animal encountered each of the above test conditions, the rats being randomly allocated to different conditions in turn. A second replicate of the experiment was carried out using the remaining four rats.

Tests were carried out as follows: starting at 16:00 h, an individual rat was placed into the start box of the test system without the barrier in place. The rat would normally explore the test system immediately and once it had passed through the start box in each direction, the barrier was put into place so that its base was 6 cm above the floor of the start box. This ensured that the rat was aware of the barrier but did not need to make any effort in order to pass under it. After the rat had passed under it in both directions, the barrier was lowered to 4 cm above the floor of the start box, thus requiring an effort to lift it. With an initial barrier weight of 110 g, this was relatively easily accomplished. After the barrier had been lifted by the rat, it was raised to a height of 6 cm and left in place overnight. The nest-box, nesting material or both, was introduced to one of the cages. The rat was then left to establish its resting place overnight. On the following morning, at least one hour after lights-on, the rat was removed by hand from the cage in which it was resting and placed into the other cage. The barrier in the start box was then lowered and a 50 g weight added to it. The apparatus was checked 3 h later, to determine whether the rat had successfully lifted the barrier and returned to its nest, If so, the rat was used in a further trial by being moved to the other cage and the weight of the barrier being increased by 50 g. Three trials could be completed in one day, and these were repeated using progressively heavier weights on the barrier, until either the rat would no longer lift the barrier to return to its resting place, or until a maximum barrier weight of 460 g was reached. Thus rats lifting the maximum weight would have undergone eight trials:

barrier only (i.e. 110 g)
barrier + 50 g
barrier + 100 g
barrier + 150 g
barrier + 200 g
barrier + 250 g
barrier + 300 g
barrier + 350 g

Such trials took 3 days to complete, and the rats were allowed free access to the whole system overnight, between the test days. Video recording of the trials was carried out while the experimenter was out of the room, because in some cases the rats were found to have squeezed under the barrier, returned to the nest and later emerged and gone back to the other cage to feed. Without video recording, such a result could have been registered as a failure to lift the barrier.

The rat was returned to its home-cage at the end of each test. The remaining tests were carried out as described above, the rat being allowed to acclimatize to the test system and to establish a resting place overnight before testing began.

Analysis

Due to the limitations of the equipment, 460 g was the maximum weight that could be lifted; however the likelihood is that the rats could have lifted more. Thus, the maximum weight that a rat would lift in each category was treated as a failure weight, and the data analysed using a stratified Log Rank test within SAS procedure Proc Lifetest (Kalbfleisch & Prentice 1980). The stratification by rat adjusts the analysis for variation between rats in responses. This analysis assesses the dependence of weight lifted upon the presence of nesting material, nesting box and the interaction of these two factors. The strength of preference Log Rank model used was:

$$\text{Weight lifted} = a + bNB + cNM + dNB{*}NM + \text{error}$$

where the model coefficients are: a = intercept; b = the main effect of adding the nest-box; c = the main effect of adding the nesting material; d = the interactive (synergistic) effect of adding nest-box and nesting material together.

Results

One rat failed to lift the un-weighted barrier in order to pass through the centre box in the initial training session, and was replaced by another in the operant trials. All eight rats used chose to rest in the nest-box, nesting material or nest-box and nesting material, rather than to rest in an empty cage. When there was only a choice between two empty cages, the rat usually chose one in which to settle before the end of the dark period. After being moved out of the chosen resting place, the rats normally attempted to lift the barrier and return within 30 min. The rat was then able to rest for at least 2 h and 30 min before the next trial. If video recording showed that the rat had not attempted to lift the barrier, it was left in the empty cage until the end of the trial (3 h).

Table 1 **Mean weights lifted by rats required to lift a weighted barrier in order to reach a previously established nest site**

Nest site	Mean weight lifted to reach nest site (g)
Empty cage	150.0
Nest-box	327.5
Nesting material	290.0
Nest-box plus nesting material	429.0

The rats used two different strategies to lift the barrier; one was to insert the head and neck under the barrier and then to rapidly push the barrier up by several centimetres with the neck. The other was to forcefully squeeze the whole body under the barrier, resulting in only 1 or 2 cm of lift. The mean weights lifted to reach the empty cage, the nest-box, nesting material or both nest-box and nesting material were 150 g, 327.5 g, 290 g and 429 g respectively (Table 1). Analysis by Log Rank test showed that the weight lifted in these trials was significantly increased by the presence of a nest-box as compared with an empty cage ($P = 0.002$), but that the increase in lift weight for nesting material alone did not quite reach statistical significance ($P = 0.078$). The interaction between the effect of nesting material and nest-box was not significant ($P = 0.966$), indicating that adding both to a cage had no more effect than the sum of the two individual effects.

A considerable degree of variation in the weights lifted was found between the rats used in this study. However no correlation was found between the total weights lifted by each rat and their individual body weights (Fig 3).

Discussion

The results of these operant tests were concordant with previous findings that rats preferred cages furnished with opaque nest-boxes or paper nesting materials to unenriched cages. Similar preferences have been found among mice (van der Weerd *et al.*

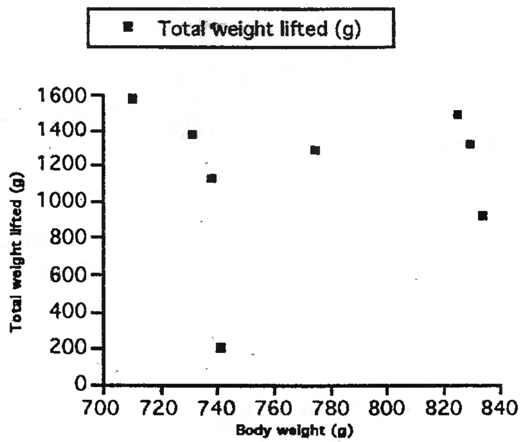

Fig 3 Correlation between body weight and total weight lifted by individual rats in operant trials

1996*a*). Although there was no synergistic effect between the nest-box and the nesting material, both items appeared to increase the attractiveness of the cage to which they were added. The rats used in these trials were prepared to make a considerable effort to return to a furnished nest site and were able to discriminate between different nest sites. However, the difference in effort made to reach an enriched cage as compared with an empty cage was significant only for cages containing the nest-box. At first sight, this indicates that the rats preferred nest-boxes to nesting materials. However, it may be significant that testing was carried out during the light period only. Despite a relatively low light intensity in the test area, the rats may have worked harder to reach the nest-box in order to escape the light. Further tests to determine the preference during the dark period would be useful. It is interesting to note that mice, when offered a choice between cages containing nest-boxes and nesting material, did not carry material from cage to cage. Over a 24-h period, they showed a preference for nesting material rather than nest-boxes (van der Weerd *et al.* 1996*b*).

On the whole, the rats rapidly accepted the test apparatus and eight out of nine rats completed the operant trials. The spring-loading device was found to be a valuable modification to the operant testing system since it ensured that the barrier could not fall on the rat, as was sometimes observed by Manser *et al.* (1996). The rats used in the current study were, on average, 38% heavier than those used by Manser *et al.* (1996) and this may explain why many of them were able to lift the maximum weight possible in the apparatus.

Since the rats had to be moved from one cage to another by hand at least three times a day, it was essential that they did not react strongly to handling. The amount of handling (a few minutes once a week by the experimenter and once a week by technicians) appeared to be sufficient to maintain docility in these rats. Similar handling has previously been found to be effective in rats of the same strain and used in preference and operant studies, but housed in another facility (Manser *et al.* 1995, Manser *et al.* 1996).

This study confirmed previous findings that laboratory rats do prefer cages furnished with either a nest-box, nesting materials or both, to empty cages. It also showed that rats are prepared to work in order to reach cages so furnished. Both items require little extra in terms of labour or financial outlay, and are suitable for animals used in toxicological studies. This indicates that the provision of nest-boxes and nesting materials is an effective way of enriching the cage environment of laboratory rats. SmithKline Beecham Pharmaceuticals are now implementing more widespread use of this proven design of nest-box in their facilities. An evaluation and comparison will also be made with commercially available autoclavable smoked polycarbonate cages, cut into sections across their long axes. ('ULTEMP', Allentown Caging Equipment Company Inc., Route 526, PO Box 698, Allentown, New Jersey, USA)

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