253. Manser, C.E., Elliott, H., Morris, T.H. and Broom, D.M. 1996. The use of a novel operant test to determine the strength of preference for flooring in laboratory rats. *Lab. Anim.*, 30, 1-6.

The use of a novel operant test to determine the strength of preference for flooring in laboratory rats

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Summary

A previous study showed that laboratory rats preferred to dwell on a solid floor rather than a grid one, particularly when resting (Manser *et al.* 1995). The strength of this preference was investigated in an operant trial using a novel test apparatus, which consisted of a grid-floored cage and a solid-floored cage, joined via a central box containing a barrier whose weight was adjustable. Trials in which rats had to lift the barrier in order to explore the whole apparatus were alternated with those in which the rats were confined on the grid floor and then had to lift the barrier in order to reach the solid floor. The latter trials were carried out at the beginning of the light period when the rats were seeking a resting place. In both trials, the weight of the barrier was progressively increased for each rat, until a maximum weight was found which it would lift either to explore its environment (weight A) or to reach the solid floor (weight B). No significant differences were found between weights A and B, showing that rats would work as hard to reach a solid floor to rest on as they would to explore a novel environment. The apparatus used could, with some modifications, be appropriate for use in other operant studies in laboratory rats.

Keywords Laboratory rat; grid floors; caging; animal welfare; preference; strength of preference; animal behaviour, operant testing

Provision of appropriate flooring in cages for laboratory animals has important consequences for welfare. The previous part of this study (Manser *et al.* 1995) indicated that rats prefer to dwell on a solid floor rather than a grid one, especially when resting. In particular, rats which were given a choice of a solid or a grid floor were observed to seek out a resting place on a solid floor soon after lights came on. However, the finding that rats preferred to rest on a solid floor is merely a qualitative result. Indeed, preference tests have been criticized because they only 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). An examination of the literature showed that although there have been several studies in which the preferences of laboratory rodents for certain commodities have been investigated (see Manser *et al.* 1995), there have been no attempts to quantify the strengths of those preferences. It was therefore necessary to find a method of testing how strongly rats preferred to rest on solid floors.

One method of determining the strength of preference for a particular commodity is to use operant tests to show how hard an animal will work to obtain it. For example, Duncan & Kite (1987) measured the force which

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chickens would exert by pushing against a door in order to reach a nest box. This method was later refined and validated, in order to demonstrate the degree of motivation of chickens to reach food after different periods of food deprivation (Petherick & Rutter 1990).

The use of an appropriate operant for testing the strength of preference is important; Dawkins & Beardsley (1986) showed that hens would not peck a coloured key in order to reach litter although other testing methods had indicated their strong preference for litter. Similarly, although many experiments have been carried out in which rats are taught to press a lever or turn a wheel in order to reach a goal such as food or flavoured water (Silverman 1978), they might not associate such activities with reaching a solid floor. A method which required the rats to make a direct effort to reach their goal, by pushing or lifting, was considered more appropriate. We therefore proposed to determine whether rats would lift a weighted barrier in order to reach a solid floor when they wished to rest.

During pilot trials with the apparatus, the rats were seen to lift considerable weights in order to explore it, presumably because of the novel environment which it provided. This is not surprising since many other studies have shown that rodents are strongly motivated to explore novel environments (Cowan 1983). The motivation of the rats to explore the apparatus therefore appeared to be a relevant standard with which to compare their desire to reach a solid floor. It was decided that the weight which rats would lift in order to reach a solid floor for resting would be compared with that lifted so as to explore a novel environment.

Materials and methods

Animals and housing

The animals used were 8 male Sprague–Dawley rats[1], aged 5 months, which had all shown a preference to rest on a solid rather than a grid floor, in choice tests carried out previously (Manser *et al.* 1995). The rats were purchased at 3 to 4 weeks old, since when 4 had been housed in cages with solid floors, supplied with sawdust bedding[2] and 4 had been in cages with stainless steel grid floors (wire diameter 1.9 mm; mesh size 7.5 mm × 7.5 mm) suspended over trays containing the same bedding. The animals were kept in pairs in polypropylene cages measuring 56 cm × 38 cm × 20 cm[3] and housed under barrier conditions with ventilation under positive pressure, with temperature and humidity controlled at 21°C ± 2 and 50% ± 10. They were fed *ad lib* on a complete expanded diet[4], mains water was available *ad lib* from water bottles and all rats were handled briefly twice a week. The light:dark period was 12:12; lights on 10:00 to 22:00 h. This facilitated study of the rats at the time the lights were switched on and ensured that the tests occurred when there was not a great deal of other activity in the animal house. The light intensity was 100 lux in the main part of the room. The normal light intensity used in rat rooms in this facility was 350 lux, but pilot tests showed that the rats were reluctant to emerge from under the food hoppers in the test cages at this level of intensity. During the dark period, a dim red light (2 lux at 'rat level') was illuminated, enabling video recording.

Test apparatus

The operant testing system consisted of 2 polypropylene cages, similar to the home cages, one with a grid floor (cage G) and one with a solid floor (cage S), each being joined by tubes to a central box containing an adjustable barrier. The central box measured 30 cm × 15 cm × 19.5 cm high and the tubes were 8.5 cm in internal diameter. The connecting tubes, central box and barrier were all made of transparent perspex. The 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 (see Fig 1). Two test systems were used at a time

[1]Virus antibody free CR1: CD BR rats. Charles River UK Ltd, Margate, Kent

[2]Goldchip sawdust. Special Diets Services Ltd, Witham, Essex

[3]RC2 cage. North Kent Plastic Cages Ltd, Erith, Kent

[4]R&M1 SQC(E). Special Diets Services Ltd, Witham, Essex

and they were placed on the floor of 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, so that the rats had no visual contact with each other and only minimal auditory and olfactory contact.

Experiment 1 To compare the amount of weight a rat will lift in order to reach a solid floor with that which it will lift in order to explore a novel environment

First exploratory test

Starting at 14:00 h, a rat was placed individually into the test system with the barrier set at a height of 6 cm, allowing awareness of the barrier but not requiring any effort to pass under it. After the rat had passed under it in both directions, the barrier was lowered to 4 cm, requiring an effort to lift it. With an initial barrier weight of 110 g, this was relatively easily accomplished. After a further passage in each direction, the weight of the barrier was increased by 40 g. Subsequently, each time the rat passed under the barrier in both directions, the weight it was required to lift was increased by a 40 g increment. The final weight was recorded as W1 g. Each trial lasted 15 min, after which time the barrier was raised to 6 cm, and the rat was left in the test system until the following morning. Two rats which had not started to explore the system within 15 min were not used again, and they are not included in the total of 8 rats previously described.

First flooring test

On the morning after the exploratory test, each rat was tested to determine how much weight it would lift in order to reach the solid floor when the light period began. One minute before the lights came on, the barrier was lowered to 4 cm and weighted at W1 g. If the rat was in either cage S or the centre box at this time, it was placed into cage G. Within 2 or 3 min most rats would attempt to lift the barrier and enter cage S. If a rat lifted the barrier and settled to rest on the solid floor then it was allowed approximately 30 min to rest before being returned to its home-cage. If the rat reached cage S but then returned to cage G, the weight of the barrier was increased to W1+40 g. If the rat lifted the heavier weight in order to re-enter cage S, it was then allowed to rest here for 30 min before being returned to its home-cage. The final weight (W2) lifted to reach the solid floor was recorded; unless the rat had returned from cage S to cage G, then back to cage S, W2 was the same as W1.

The centre box and barrier were thoroughly washed in hot water before the next trial and test cages were autoclaved between use by each rat.

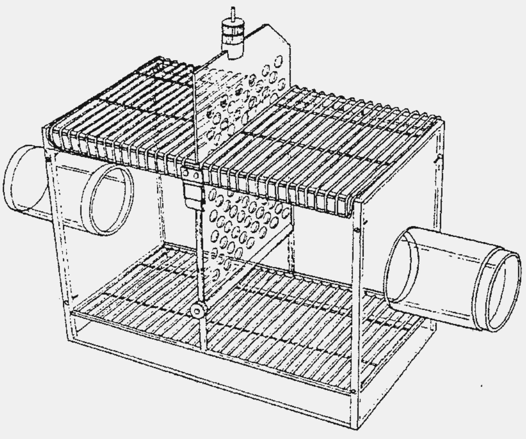

Fig 1 Centre box used in operant testing, containing a central barrier of adjustable weight and height from the floor. The box, barrier and connecting tubes are made of perspex, and the floor and roof are made of steel wire mesh. The connecting tubes allow the box to be joined o a cage on either side

Subsequent trials

Since the cages were thoroughly cleaned and re-bedded between trials, so that the environment was novel in terms of olfactory cues, the rats were motivated to explore when put back into the test system. During the second exploratory test, the barrier was initially raised until the rat had passed under it in both directions. Subsequently after a passage in each direction, the barrier was lowered to 4 cm and un-weighted. After a further passage in each direction, the barrier was weighted at W2+40 g and the weight increased by 40 g increments every time the rat had passed in both direction. The rat was allowed 15 min to explore the system and the final weight lifted was recorded. As before, the barrier was left raised overnight. On the following morning at lights-on, a flooring test was carried out as before except that the first weight added to the barrier was equal to that lifted in the exploratory trial on the previous day.

Thereafter the rats were re-tested in this way on subsequent days, so that an exploratory test in a clean cage was followed by a floor test the following morning at lights-on. Trials proceeded with progressively increasing weights applied to the barrier, but in the later stages when the rats were struggling to lift it, the weights were increased by 20 g rather than 40 g increments. When a rat had refused to lift a particular weight in an exploratory test, it would be tested with the same weight in a flooring test and vice-versa. Eventually a maximum weight was found which each rat would lift both to explore (WE_{max}) and to reach the solid floor (WS_{max}).

Results

All of the rats lifted a weighted barrier to explore the novel environment provided by a clean test system, and to reach the solid floor on the morning of the floor tests. The rats were frequently observed to struggle in their attempts to lift the barrier, especially when it weighed over 300 g. As the weights were progressively increased, each rat would eventually encounter a weight which it appeared unwilling or unable to lift. The number of trials undergone by individual rats before a maximum weight was reached in each type of trial ranged from 4 to 10. Some rats appeared reluctant to pass under the barrier as its weight increased whereas others would struggle persistently to lift it; not only did the barrier become more difficult to lift as its weight increased but some rats appeared nervous because it then tended to drop rapidly after they had passed under it. The rats which persisted in lifting the barrier and therefore took part in more trials tended to lift the greatest weights but the weight lifted did not appear to be correlated with body weight (see Table 1).

Table 1 Details of rats used in operant trials

Rat	Body weight (g)	Maximum weight lifted (g)	Number of operant trials[1]
G104	520	430	10+10
G106	530	410	10+10
G113	524	310	9+9
G116	544	170	4+4
S104	528	310	7+7
S107	639	310	6+6
S111	574	190	5+5
S113	608	360	8+8

[1]Number of trials for exploration and for reaching the solid floor

However in all cases the weight which each rat was able or willing to lift either to explore the test system or to reach the solid floor at lights-on was similar (see Table 2). Comparing within-animal results by means of a paired 2-tailed *t*-test, there were no significant differences between the 2 weights lifted. Thus it appears that the rats were prepared to make as great an effort to reach the solid floor at lights-on as they were explore a novel environment. As can be seen in Table 2 the weights lifted by the rats were quite considerable, constituting up to 83% of the rats' own body weight. No significant differences were found between the weights lifted by rats reared on grid floors and those reared on solid floors.

Table 2 Weight lifted by rats in operant trials

Rat	Maximum lifted in exploring (WE_{max}) (g)[1]	Maximum lifted to reach solid floor (WS_{max}) (g)[1]
G104	430	430
G106	410	410
G113	310	290
G116	170	150
S104	280	310
S107	310	310
S111	190	150
S113	360	360

[1]No significant difference between the 2 columns of results using a 2-tailed paired t-test

Table 3 Numbers of times rats finally rested in cages G or S respectively, in Experiment 2

	Rats starting in cage S	Rats starting in cage G
Number of times rats finished in cage S	29	29
Number of times rats finished in cage G	8	10

The preference for finishing in cage S, regardless of the start cage, was statistically significant ($P<0.05$)

Experiment 2 To determine whether rats confined on a solid floor will lift a weight in order to reach a grid floor

The rats were inquisitive about any manipulation of the barrier from outside the test box and tended to investigate the barrier shortly afterwards, sometimes passing under it. It was considered that in Experiment 1, if the rat were placed in cage G shortly before the lights came on and the barrier was then lowered and weighted, that the passage of the rat through to cage S might be purely an investigative action. Therefore the same rats as in Experiment 1 were subjected to further trials, starting either in cage G before lights came on or in cage S.

The rats were left individually in the test system overnight, with the barrier raised to 6 cm. In the morning, shortly before the lights came on, the rat was placed alternately in either cage S or cage G with the barrier in the centre box lowered to 4 cm. In order to take into account the variation in weight lifted by the rats, the weight of the barrier was set at half of the maximum weight which the individual had lifted in Experiment 1. Each rat was tested 10 times, starting in cage S on 5 occasions and in cage G on the remaining 5 occasions.

Results

On the majority of occasions, the rats settled on the solid floor when the lights came on; this was true regardless of whether they were placed in cage G or cage S just before lights-on. On 4 occasions, data were lost so the total figures for final resting places are 39 for rats starting in cage G and 37 for rats starting in cage S (see Table 3). Rats reared on grid floors finally rested on a solid floor on 27 out of 37 occasions, and rats reared on solid floors finally rested on the same type of floor on 31 out of 39 occasions. This shows that although the former group had now been kept on grid floors for over 6 months, they still generally chose to rest on solid floors when the opportunity arose.

The results were analysed using a generalized linear model, using binomial errors and the logistic link function. The effect of previous housing, starting cage and the interaction between these 2 factors was found not to be statistically significant, using an F-test (P=0.4980, P=0.6036, P=0.4348, respectively). Therefore the model used was:

$$\log(\pi_1/(1-\pi_1))=\beta_0+E_b$$

where π_1 represents the proportion of occasions the rat chose cage S, β_0 represents a constant, and E_b represents the error between animals.

The overall estimate for the proportion of occasions on which rats chose cage S rather than cage G was 0.763 (limits of error 0.566–0.888). If there had been an equal preference for cage S and cage G at the 95% confidence interval, then this interval would have included 0.5. Since this was not the case, the preference for cage S is statistically significant ($P<0.05$).

Discussion

The apparatus devised for this study proved inexpensive and was relatively simple to assemble and use. It also provides an effective method of determining the effort which a rat would make to reach a desired goal. The weights lifted by individual rats showed a large degree of variation which was not related to body weight. As previously noted, rats sometimes appeared to be alarmed by the rapid dropping of the barrier behind them as they passed through the centre box. This would sometimes inhibit the rats from attempting to pass through again. Some type of counter-balancing system to prevent the barrier from dropping suddenly might prove to be a useful modification to the apparatus.

Since motivation to reach a solid floor at lights-on could be tested only once every 24 h and it was practically possible to use only 2 test systems simultaneously, several weeks were required to collect the data. A method for automatically raising and lowering the barrier and measuring the amount of effort made by the rats to pass under the barrier would greatly enhance the precision of data collection and would permit the simultaneous use of several test systems. Automation would also reduce the risk that rats might be influenced by the presence of an experimenter.

The apparatus used in this experiment could be useful in other studies where the strength of preference in rats is to be determined. The rats were willing to work to lift the barrier throughout the trials and there was no evidence of habituation. However, it is possible that a point might be reached where the rats would no longer make a great deal of effort in order to explore the test system. In this case, another method of motivation, such as provision of a favoured food pellet beyond the barrier, would be required.

The rats appeared to be highly motivated to explore the novel environment provided by a recently cleaned test system. This strong motivation to explore has frequently been observed in laboratory rats (Cowan 1983) and the possible functions of this which have been suggested are simple curiosity, or the seeking of either hiding places, escape routes to a more familiar environment or a return to their normal companions (Russell 1983). The latter could be significant in this experiment, where rats in the test system were separated from their usual companions. This motivation to explore is likely to be important for survival; as stated by Cowan (1983): 'Exploratory behaviour enhances the survival of individuals in 2 ways, by reducing factors (such as vulnerability to predation) influencing mortality and by increasing factors (such as foraging ability) which contribute directly to survival'.

These experiments showed that the rats studied were also strongly motivated to reach a solid floor when they wished to rest. The results from Experiment 1 indicate that the rats would lift up to 83% of their bodyweights in order to explore a novel environment or to reach a solid floor. The fact that rats were apparently willing to make such considerable efforts to lift the barrier suggests that they might find this effort rewarding in some way. However the results from Experiment 2 showed that they were more likely to go from a grid floor to a solid floor as lights-on than *vice versa*, and that they chose to rest on the solid floor, whichever floor they started on. The experiments therefore confirm that, as indicated in Manser *et al.* (1995), rats do prefer to rest on a solid rather than a grid floor. Taking the results of both papers together, there was no evidence that the health of rats housed on grid floors was impaired or that their behaviour was significantly different from those housed on solid floors. Both studies indicated that rats prefer to rest on solid floors. The experiments reported in this paper also show that rats are prepared to make considerable efforts to reach a solid floor when they wish to rest. Thus there are grounds for suggesting that laboratory rats be housed on solid rather than grid floors whenever possible.

Acknowledgments We wish to thank Ms J. Latcham and Ms K. Prescott for providing high quality animal care and valuable assistance with experiments, and also Mr A. Long for building the apparatus used for operant testing. We are also grateful to Dr H. B. Waynforth of SmithKline Beecham Pharmaceuticals for his advice on the experimental design used in this study.

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