USE OF IN-CAGE SHELTERS BY LABORATORY RATS

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The effect of providing a shelter to single-housed rats was measured in terms of the preference shown for a cage containing a shelter compared with a barren cage, the range of behaviours performed and the apparent fearfulness of the animals. All animals showed a strong preference for cages containing a shelter and rats housed in this environment showed increased exploratory behaviour and were apparently less fearful, based on their willingness to leave the home-cage. It would seem appropriate to provide enclosed shelters within the cages of laboratory rats as a way of improving their environment.

Keywords: *animal welfare, enrichment, housing, rats, shelters*

Introduction

Many national and international bodies have drawn up codes of practice or recommendations with respect to the housing of laboratory animals (Canadian Council on Animal Care 1984; US Department of Health and Human Services 1985; Council of Europe 1986; Home Office 1989, 1995). These reports mainly address aspects of the animals' physical environment. However, attention is now turning to methods of environmental improvement, in order to address the animals' psychological well-being as well as their physical condition.

The European Commission's international workshop on the *Accommodation of Laboratory Animals in Accordance with Animal Welfare Requirements* (Brain 1995) stated that 'cages for laboratory rodents should satisfy the physiological and ethological needs of resting, grooming, exploring, hiding, searching for food and gnawing'. In addition it recommended that 'there should be room for hiding or escape from conspecifics within the cage' and that 'attempts should be made to make the cage more utilizable by structural divisions'.

With respect to the physical environment, the report emphasized that 'the microclimate within the cage is what is really important to the animals and welfare seems improved when rodents have a degree of control over it' and 'animals should be given the opportunity to withdraw to shaded areas'.

The *Code of Practice for the Housing and Care of Animals in Designated Breeding and Supplying Establishments* (Home Office 1995) also discusses aspects of housing for rodents, mentioning the value of environmental improvement in breaking up the cage to provide complexity, giving as examples the use of corrugated devices or tubes which can also act to increase available floor area. Indeed the provision of cage enrichment is 'considered to be of more value to the animal than simple floor space allocation' by the authors of this report.

Thus, it is possible to identify aspects of the animal's environment which can be manipulated in ways that may improve the welfare of the animal. In this study, experiments were devised to test the following hypotheses:

1) That rats will show a preference for using a shelter-enriched environment when given a choice between that and a barren one.
2) That rats housed in a shelter-enriched environment will show less fear than those housed in a barren environment.

Materials and methods

Animals

Twenty outbred male rats of the Wistar stock were purchased from Charles River UK Ltd, (Margate, UK). They weighed 150g on arrival and were specified as VAF/Plus, ie free from all microbiological agents listed in the Federation of European Animal Science Associations' (FELASA) recommendations (FELASA Working Group on Animal Health 1994). They were transported from the supplier by road and allowed to acclimatize for a minimum of three weeks prior to use in the experiment.

Housing

Animals were housed individually in transparent Makrolon RC4/F cages (North Kent Plastic Cages Ltd, Erith, UK) of overall dimension 590x380x220mm, in an animal holding room which was supplied with 25 air-changes per hour (non-recirculated). Lighting was 12 hours dark, 12 hours light, lights coming on at 0730h and going off at 1930h. Light intensity varied from top to bottom of the rack, but averaged 48 lux in the cages. Animals were provided with sawdust bedding material (Grade 1200, Datesand Ltd, Cheshire, UK) which was changed, along with the cage, every 7 days. Animals were given ad libitum access to RMI(E) diet (Special Diets Services, Wrotham, UK) and water. Water was renewed every second day and water bottles changed weekly. The room temperature was maintained at 21°C ± 1°C and relative humidity within the room was 55 per cent ± 10 per cent.

Half of the animals were given 'enrichment', which consisted of an upturned M2 polypropylene mouse cage (North Kent Plastic Cages Ltd, Erith, UK) of overall size 330x150x130mm, placed in the centre of the RC4/F cage with the opening facing the food-hopper. These were designated as the 'C' group. The remainder of the animals were designated the 'B' group.

The cages were placed on two racks alternating the positions of those given enrichment with those lacking enrichment so as to control for the effect of rack position.

Experimental procedures

There were four experiments performed in the study.

Home-cage behaviour

On each occasion four animals were taken from the holding room to the experimental room in their home-cages for recording of their behaviour.

The experimental room was an animal holding room with the same environmental parameters as described above. Animal cages were placed on the floor. With room lights on, the in-cage light intensity was 40 lux. During the dark period, a red light was left on to

provide low level illumination (average in-cage light intensity 2 lux). Animal activity and behaviour were observed using a Panasonic television camera and recorded real-time on to videotape using a Panasonic video recorder.

The video recorder was within the experimental room but was shielded from the cages by a hardboard screen. Ultrasound measurements were taken within the room using a Mini-2 Bat Detector (Ultra Sound Advice, London, UK) with the camera, monitor and video recorder switched on. No noise was detected from the camera. Noise from the television monitor was evident but this ceased when it was switched off, as it was throughout all experimental sessions. Ultrasound was emitted by the video recorder but this was not detectable from the area of the cages when the recorder was shielded.

Cages were visually separated from each other by two wooden barriers. This arrangement formed an effective partition between the four cages and also reduced olfactory and auditory communication between occupants of the different cages.

Cages were placed in the experimental room at 1400h on each experimental day and behaviour was recorded for a continuous 8-hour period starting at 1730h or later, such that the entire period 1730h to 0930h was covered in the course of the experimental period.

As each experimental session consisted of two animals from both B and C groups, the times of recording were the same for each group.

The position of the B and C cages in the experimental grid was adjusted such that cages were alternatively to the left and right of the camera in both near and far positions. This was to allow for the possibility that behaviour may have been affected by cage position on the floor. Each animal's behaviour was recorded for one session only.

Preference testing

The preference testing system consisted of two Makrolon RC4/F cages, joined together as described in the following: at the end of each cage farthest from the food hopper, a circular hole was made of diameter 80mm. A piece of opaque PVC tubing 80mm long and diameter 80mm was placed into the hole, forming a connection between the cages. Each cage was identical in that they were each supplied with food, bedding and water in the same amounts. One of the cages contained an upturned M2 mouse cage, reserved specially for use in the preference test.

Between each test, the cages and inserts were broken down and washed in an automatic cage washer for 3 minutes at 82°C using a detergent and bactericide (Utex & Bactirinse, Sychem UK Ltd, Middlesex) to ensure removal of odours.

Animals were transported to the experimental room in their own cages at 1400h on the day of the experiment and left in their own cages until 1730h, when they were placed into the preference system. One animal was placed in each system and the two systems were used simultaneously. Each was used to test a B and a C animal at the same time.

Each test was started at 1730h when an animal was placed into the half of the preference system which was similar to its home-cage. Animals were left to acclimatize to the system for a minimum of half an hour prior to recording. Recordings were continuous for 8 hours. Half of the recordings commenced at 1800h and the other half at 0100h, so that the period covered was 1800h to 0900h inclusive, ie the most active period of behaviour (Batchelor 1994). Each animal was tested in the preference system on one occasion only.

Video recordings were analysed by noting the position and behavioural activity of the rat within the system every 10 minutes using the scan sampling method (see Altmann 1974). This behaviour was classified using an adaptation of the ethogram by Grant and Mackintosh (1963) into one of those listed in Table 1.

Food and water consumption were measured in both ends of the preference system for the period that each animal was in the system. Total food and water consumption was then calculated.

Home-cage food consumption

It was noted through the initial weeks of the experiment that there seemed to be an increased food consumption in the barren cages. To test this observation, at the end of the home-cage and preference experiments, all animals were left for one week in their cages in the holding room. Sixty grams of food was measured out to each animal. Food was weighed daily at 1030h for 10 days and the amount consumed in the previous 24 hours calculated. The amount of food for each rat was made up to 60g after weighing on each day. The total consumption of the B and C rats over the 10-day period was calculated.

Home-cage emergence

To assess whether there was any difference in fearfulness between animals housed in cages with a shelter and those without, at the end of the 'home-cage food consumption' experiment animals were tested separately for their readiness to emerge from the home-cage. The experiment was performed between 1000h and 1200h within the animal holding room. The animal's home-cage was placed on the floor in the middle of the room, the lid removed and a stopwatch started. The observer then left the room and observed the animal through a window in the door. The stopwatch was stopped when the animal put all four feet on the floor outside the cage.

Statistical analysis

The frequency of each home-cage behaviour was noted for each animal and the totals for all animals in each cage-type calculated to give a cumulative measure. Differences in frequencies of behaviours between groups were tested for using the Mann-Whitney *U* test.

In the preference tests, simple observations of where the animals were at 10-minute intervals throughout the recording period were made and frequencies of presence in each side of the system were calculated. Resulting data were analysed by analysis of variance for differences in side preference between those animals kept in barren cages and those in enriched cages.

Consumption of food was calculated and differences between groups tested by analysis of variance.

Time to emerge from the home-cage was measured and differences between cage-types were analysed by analysis of variance.

Results

Home-cage behaviour

Because of the presence of the shelter in C cages, the range of behaviours involving this item were obviously not expressed by the B cage animals. However, if the 'rest' and 'explore'

behaviours involving the shelter were added for group C then there was a significant difference ($P < 0.001$) between the groups in the amount of time spent exploring (Table 1). The C group spent more time in exploratory behaviours than the B group, which conversely spent more time resting, ie inactive, although the difference here was not significant.

There was a significant difference ($P < 0.001$) between groups in 'rear' and 'walk', group B performing these behaviours more than group C. There were no significant differences between the groups for other behaviours.

Table 1 **Frequencies (and percentages of total behaviour observed) of home-cage behaviours for rats housed with (group C) and without (group B) a shelter. A total of 320 observations were made for each group.**

Behaviour	Frequency of behaviour	
	Group B	Group C
Rest	256 (79.9%)	14 (4.4%)
Rest in box	-	217 (67.7%)
Rest on box	-	18 (5.6%)
Explore	12 (3.8%)	20 (6.3%)*
Explore on box	-	33 (10.3%)*
Walk	16 (5%)	3 (0.9%)*
Rear	14 (4.4%)	3 (0.9%)*
Groom	5 (1.6%)	4 (1.3%)
Feed/drink	17 (5.3%)	8 (2.5%)

*indicates significance at $P < 0.001$

Preference testing

There was a significant difference ($P < 0.001$) in the preference of both groups for the enriched side of the preference system over the barren side (Table 2). When data from the groups were combined there was an overall significant preference for the side of the system containing the shelter ($P < 0.001$). There was no difference in preference between animals in the system nearest the camera and those further away from the camera.

Table 2 **Percentage preference of rats housed with (group C) and without (group B) a shelter for barren versus enriched side of the preference system.**

System	Percentage preference		
	Group B	Group C	B and C
Barren side	10.9	10.3	10.6
Enriched side	89.1	89.7	89.4

All preferences for enriched cages were significant at $P < 0.001$

There was no significant difference in water consumption between either end of the preference system (Table 3). However, there was a significant difference ($P < 0.05$) in food consumption by rats between the barren and enriched sides of the preference system, with more food being consumed in the barren end of the system than in the end containing the shelter.

Table 3 **Food and water consumption (mean and SEM) of rats housed with (group C) and without (group B) a shelter when placed in the preference system.**

Consumption by side of system	Group B rats	Group C rats	B and C
Barren side			
Water (ml)	12.7 (7.2)	13.6 (5.5)	13.2 (6.3)
Food (g)	14.6 (9.2)	16.7 (10.3)	15.6* (9.6)
Enriched side			
Water (ml)	18.2 (6.8)	14.1 (5.8)	16.1 (6.5)
Food (g)	10.0 (10.0)	8.8 (10.5)	9.4* (10.0)

* indicates significance at $P < 0.05$

Home-cage food consumption

There was slightly greater mean food consumption in the barren cages as compared to the enriched cages (B mean = 296.7g ± 38.7 (SEM) and C mean = 277.6g ± 32.9 (SEM)). However, this difference was not significant.

Home-cage emergence

Mean time to emerge from the home-cage for the B group was 507s ± 224.6 (SEM) and for the C group it was 173.7s ± 224.6 (SEM). Animals housed in C cages were significantly quicker to emerge from the home-cage ($P < 0.001$).

Discussion

The provision of a shelter gives the rat a number of potential environmental stimuli which allow it to perform an increased range of behaviour, viz: 1) an enclosed space to satisfy the thigmotactic aspect of the animal's behaviour; 2) a hiding place for avoidance of conspecifics; 3) a platform for climbing; 4) a shelter in which the microclimate is different to that outside, eg lower light intensity, higher temperature and humidity. At the same time, the larger cage still allows the animal to move around and rear on its hind legs (Lawlor 1984).

In addition it may be argued that the provision of a shelter within the cage adds to the complexity of the animal's environment. Work by Denny (1975) and Chamove (1989) indicated that both mice and rats seem to show a marked preference for environmental complexity, which Denny (1975) interpreted as a shelter/relaxation-seeking response. Thus, the provision of a shelter may enable the animal to meet a behavioural 'need' for complexity in its environment. In addition, Chamove (1989) reported that mice kept in structurally and spatially complex environments were less fearful than those kept in barren cages.

Boyd (1988) and Ward and DeMille (1991) provided refuges for mice using water bottles. These were used both as shelters and as latrines by the mice. In this study, there was no obvious use by the rats of the shelter as a latrine area, possibly because it did not have a floor. Shelters with a floor could be constructed to test whether rats would use this design in a similar way to mice.

The greater amount of exploratory behaviour observed in animals kept in the enriched cages may be explained by the fact that the M2 cage provides an object on which the animal can focus its attention, in what is otherwise a relatively barren environment. Also, the rats in this group were able to climb on to the M2 cage and use it as a platform, which enhanced their ability to explore. This increased amount of exploratory behaviour by animals in the enriched cages may also represent a decreased fearfulness in these animals compared to those kept in a barren environment.

At the end of the experimental period many of the mouse boxes were found to have been extensively chewed. As rodents are by definition chewers, this should not be surprising. One area for concern which arises from this finding is the possibility of toxicity or physical obstruction due to ingestion of fragments of the plastic. There was no evidence of any health problems in the C group throughout the experiment although no post-mortems were carried out on these animals to check for any chronic problems. During the time this method of enrichment has been in use at this Institute (>1 year), no problems attributable to cage chewing have been recorded. The fact that the C group consumed less food than the B group may be explained by the focus of their chewing behaviour being redirected somewhat to the mouse cage. Alternatively, eating may be one of the few activities available to rats in barren cages.

The circumstances of this experiment were serendipitous in that the mouse cages used were surplus and of no value. However, in light of these observations it may be that the use of alternative, non-toxic shelters, possibly disposable, of cardboard, plastic or metal, needs to be explored in the future.

The clear preference of both groups for cages containing the M2 cage may indicate that rats have a desire for a sheltered area within the cage, greater than that which is normally available, eg beneath the food-hopper. This is clearly shown by the fact that even the B group which were familiar with the empty cages, left their familiar surroundings when a more substantial shelter was available.

Some criticisms of preference testing of this type have been made (Duncan 1978). Ultimately the test used in this experiment only gives an idea of the preference of the animals for one of two types of environment. There may be other, more preferable options, which the animal cannot choose because they are not offered. In addition the test supplies no information on the strength of the animals' preference for the C cage. An alternative method would be to devise a system in which the animal had to work for access to the shelter. The amount of work an animal would be prepared to perform in order to gain access to the shelter could then be compared to that performed to gain access to other variables, eg food-item treats. Thus a measure of the elasticity of the demand for shelter could be developed. The more elastic the demand, the less vital the animal's need for that item. An item for which demand is relatively inelastic is one which the animal will work for almost regardless of the effort expended (Dawkins 1983).

Anzaldo *et al* (1994) found that rats preferred cages where there was increased wall-surface over cages which provided opportunities to climb. This was explained in terms of rats' thigmotaxis. Whilst not directly comparable, the use of an M2 cage as a shelter does provide elements for both wall-hugging and moving in a third-dimension ie climbing.

The preference of all animals to feed in the barren end of the system may be explained by the fact that it is normal for rats to divide up space allotted to them into different areas by function. The rats may have treated the end of the system containing the shelter as 'living space' and the barren end of the system as the feeding area. The level of defecation and urination in each end of the system was not measured, but it may be that the rats were also using the B cage as a latrine. This would be an interesting area for further study.

The significant difference in home-cage emergence times may indicate that the group housed in the C cages were less fearful than those housed in barren cages. This was also noted anecdotally in that the animals from the cages with shelters were generally more easy to handle than those from the barren cages.

Animals were of a single sex and housed alone in order to remove the confounding factor of competition for use of a single shelter. Whilst the most obvious form of enrichment for rats is a conspecific, the provision of shelters for our Institutes' group-housed rats has apparently caused no problems with respect to competition between rats for access to the shelter (unpublished observations). In fact, provision of such a hiding place may provide a facility for the reduction of direct antagonistic confrontations between rats.

Another finding of this study was that some rats moved the shelters from their original positions, as well as turning them over or on to their side. Again this shows that the rat was able to manipulate the M2 cage itself to affect its own environment and maybe to provide surroundings in which it felt more comfortable.

Conclusion

The experiments showed that rats provided with environmental enrichment, in the form of shelters, exhibited more exploratory behaviour than those in a barren environment. Furthermore, rats will show a preference for a cage containing a shelter when given a choice between that and a barren one. Thirdly, the results indicated that rats housed in an enriched environment show less fearfulness than those housed in a barren environment.

It is therefore possible to conclude that, given a choice, laboratory rats will prefer cages with shelters as opposed to those without and that keeping rats in such cages may lead to animals which exhibit less fearful behaviour. However, further work needs to be done on selecting cheaper and more appropriate materials for the shelter. In addition, the size and shape of the shelter and its position in the cage could be further investigated.

Animal welfare implications

The recommendation in the recent *Code of Practice for the Housing and Care of Animals in Designated Breeding and Supplying Establishments* (Home Office 1995), which advocates the use of tubes as enrichment for rodents, should probably be amended in the light of these results which emphasize the need for an enclosed shelter.

In the meantime, old mouse boxes are recommended as a way of providing easily sanitized in-cage shelters for rats as a means to enhance their welfare.

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