Developing and testing a novel cage insert, the "Mouse House", designed to enrich the lives of laboratory mice without adversely affecting the science

D. KEY, A. HEWETT

Biological Services, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA

Summary

To embrace legal and local ethical codes, a standard environmental enrichment regime was investigated for laboratory mice bred.

This paper describes the reasoning for a permanent cage insert and the design features that were included. It describes the trial that was performed to investigate whether the insert was beneficial, prior to it being adopted. An insert, together with paper nesting material, was to be the environmental enrichment regime of choice.

Several positive, significant, findings are reported. In addition, the insert is reported as practical and easy to use by animal technicians. The cage insert, known as the "Mouse House", has been made available commercially at a low cost. Thus, each of the 3 P's of environmental enrichment (proven, practical and price) suggested in the document has been met.

The paper concludes that the "Mouse House" should satisfy requirements for both environmental enrichment and legislation suggesting the use of cage inserts that allow privacy for the animal but facilitate observation.

Key words: environmental enrichment, rodent, mice, cage insert, IVC, animal behaviour, observation.

Introduction

Why develop a novel form of mouse enrichment?

The provision of environmental enrichment for rodents is important for both legal and ethical reasons. The Home Office "Code of Practice"[1] recognises that laboratory cages should meet the physiological and behavioural needs of the animals. It recommends that animals are able to exercise and suggests that play objects be made available. The 1986 European Convention confirms this stance. Proposed European legislation, due to be enforced in 2003, suggests that cage inserts should be provided which allow privacy but facilitate observation.[2]

The potential benefits of successful environmental enrichment are overwhelming. The behavioural needs of the animals are catered for and their health and welfare is improved by as much as any other environ mental factor.[3] Indeed, the Animal Procedures Committee's report of 1994 suggests that "environmental enrichment is crucial".[4]

Whilst great strides have been made in successful enrichment in higher vertebrates, only one type of enrichment for laboratory rodents which is widely used and has been proven to be beneficial; that of nesting material.[3] We were challenged with the possibility of providing more to satisfy the rodents' physiological and behavioural needs. This was particularly apparent as "shoe box" style cages (MT1 cages North Kent Plastics Ltd.), standard throughout academia, seemed to some staff and visitors to be small and rather barren. Commercially available inserts at that time were considered to be too large for our cages, and either could not be autoclaved or were prohibitively expensive.

What were our aims?

We considered what behaviours we should try to encourage. The Universities Federation for Animal Welfare (UFAW) suggests that ways should be found of encouraging a full repertoire of normal behaviour, such as: exploring, resting, climbing, grooming, foraging, nesting and social behaviour.[5]

What behaviours should we try to discourage? Stereotyped, bizarre or disorganised behaviours, which have been identified as being the result of the inability of animals to express natural behaviour.[3] The behaviours we considered included fighting, continually looping from floor across the lid of the cage, bar biting and lethargy.

Yet, however laudable enrichment is considered, there were some basic concerns that needed to be addressed. These included: provision of standard enrichment throughout scientific studies, thus minimising variance; ensuring the animals did actually benefit and that no adverse effects were unwittingly introduced; and keeping the cost reasonable.

As animal technicians, we wanted to ensure that the insert was as practical as possible. If a cage insert is not easy to use when performing standard animal husbandry, it will not be routinely used. A cage insert therefore needs to be practical for staff to use, as well as beneficial to the animals.

We therefore set ourselves three goals for the provision of environmental enrichment to the laboratory mice at NIMR, which we have suggested as the 3 P's of enrichment:

1. **Proven enrichment.** An enriched environment for the laboratory animal, resulting in behaviours approximating more closely to those observed in the wild mouse population, where more diverse and less stereotypic behaviour is seen. The desired environment was deemed to be one that offered a mix of security and positive sensory challenges.
2. **Practical enrichment.** Suitable for use in conditions stipulated for the housing of laboratory rodents and easy for the staff to use.
3. **Price of enrichment** to be cost effective.

Why develop a cage insert rather than some other form of enrichment?

The Home Office "Code of Practice"[1] suggests that cage inserts may be more important than the size of the cage. The provision of shelters has been recognised as helpful in reducing the incidence of fighting in male mice.[6] Small rodents are adapted to shelter in safe confined areas when resting or hiding from possible external dangers. The provision of shelters within cages is therefore likely to be beneficial to their good welfare in the animal unit.

Because the case for larger cages did not seem sufficiently strong to warrant the cost of modifying or replacing our existing cages, a decision was made to add an insert into the original cage in the form of a shelter. A recent study showed that this was a popular approach. 64% of the University, Pharmaceutical and Contract sectors combined, promoted environmental enrichment by the addition of a bottle or tube.[7] We wanted to offer the cover provided by this option but we also wanted to take the opportunity of utilising other features, in an attempt to create greater environmental complexity.

Disposable shelters, made of paper pulp, are popular and certainly seem to be used by the animals.[7] However, they have not been proven to work in experimental trials. There is an ongoing cost of providing disposable cage inserts, which was considered to be too high. They obscure the animals from view, so increasing the time animal technicians need to carry out their daily visual inspections of the mice. Following a decision to introduce some Individually Ventilated Cages (IVCs), double sided racks using 1284 cage types manufactured by Tecniplast, there was also some concern about the fibres resulting from the breakdown of disposable shelters reducing the efficiency of filters. An insert that was not disposable was therefore considered to be the most appropriate approach.

The trial

In order to achieve our first goal of providing proven enrichment, we required experimental data showing the comparative ability of the insert to provide enhanced environmental enrichment.

Environmental enrichment programmes have been known to result in problems, as well as solve them.[6] When clear perspex tubes are provided to laboratory mice, there can be an increase in aggression and bar biting. We recognised that normal, natural behaviours such as eating, drinking and sleeping should not be affected. Clearly any enrichment device needs to be tested thoroughly before being used widely, in case it has inadvertent negative input.

It is also vital that the environmental enrichment provision does not threaten the integrity of the science for which the animals are being used. Specifically that food and water intake was not affected and that stress in the animals was not increased. It was recognised that a trial was needed in order to reassure our scientists that this was the case.

Providing enrichment, in any form, has cost implications. We wanted to make a business case for this investment and felt that the data from a trial would help.

The design of the cage insert

It was a practical requirement that animal technicians could observe the animals inside the insert, without having to disturb them by opening the cage and removing the enrichment article. From preference tests it was shown that mice prefer the nest box to have a dark interior.[8] We wished to incorporate provision of this "dark" interior. We elected to use transparent plastic with a red tint to it. Humans see light of wavelength 600 to 700 nanometers as red. However mice, whose eyes have retinas comprising predominantly of rods, see this light wavelength not as red, but as black – which allows for the desired effect. The activity of mice in cages illuminated by red light is exactly the same as mice in the dark.[9] Red light has, for many years, been used to illuminate the environments of nocturnal animals without disturbing them or their circadian rhythm.[10 11] The use of red light opened up the possibility of carrying out daily animal checks without the need to disturb the animals. The tinted transparent plastic utilised for the novel cage insert used in the trial, allows observation from above and from the sides.

Having access to a shelter, seen by rodents as dark, will satisfy the requirements of the Home Office's "Code of Practice" which notes that rodents' eyes are better suited to dim light conditions and suggests that areas with low levels of light are provided.[1] A high light intensity can have a deleterious effect on eyes, such as retinal degeneration, especially in albinos.[12] In a study it was found that light levels of 60 lux inside cages can cause this retinal degeneration in albino rats within 13 weeks.[13] At 32 lux, this effect would be seen over a longer period (up to three years). The recommendation was to offer a part of the cage at 25 lux.

It was also hoped that having a dark shelter, rather than a clear transparent shelter, would eliminate the aggression as reported when transparent pipes were used as enrichment devices.[6]

The basis of the insert came from considering a wild mouse nest as a shelter or retreat, then trying to make it available within a standard laboratory cage. Rodents readily use nest boxes in the wild.[14] In a study using preference testing, it was also shown that mice make choices between different cages, and that they prefer a cage containing a nest box.[3]

Domestic mice will also dig burrows and nest complexes when given the opportunity. Interestingly, the style and size of these nests are almost identical to those of wild mice, suggesting that living in such nests is a natural instinct.[15]

In an experiment investigating the behaviour of domestic Swiss mice offered an open arena, it was noted that mice readily used an artificial burrow if one was provided.[16] If a burrow was available, then the mice would explore the open arena far more than when either the burrow was not available or a burrow had not been provided. This suggests that access to a shelter makes mice more confident in their surroundings. When a model "predator" was introduced to this open arena, mice increased their use of the burrow and tended to run directly into it when the model was shown. Since laboratory mice might see animal technicians as predators, this reinforces the idea that the mice could benefit from having access to a shelter.

Nest boxes were not available as laboratory cage accessories, but plastic pipes, drinking bottles, and yoghurt pots are available as household items, and have all been reported as having some success in enriching cages.[17,18,19] We wanted to use the nest box idea but incorporate our own features, trying to obtain maximum benefit for the mice whilst making the novel insert as practical as possible for animal technicians.

The standard size of wild mouse nests is 8cm x 10cm, so giving a floor area of 80cm^2, and a height of 6cm.[15] The size of our novel insert is similar, having an internal floor area of 77cm^2, and a height of 6cm.

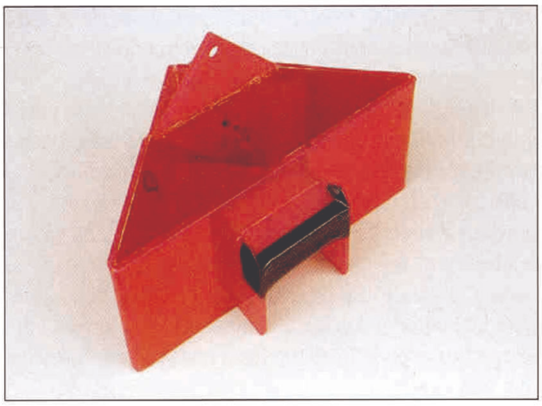

Figure A. The "Mouse House"

The shape is triangular to fit into a cage corner, at the other end of the cage to the food hopper. This is to: maximise the amount of useable open floor area outside of the insert; make it very easy to locate the insert into the best position; ensure it is well away from the bottle spout to prevent it from causing water leaks. Since the cage abuts the insert on two sides, it tends not to move within the cage.

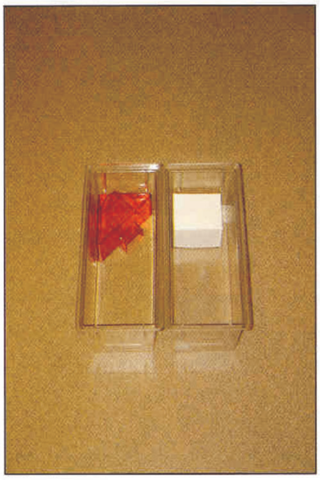

Figure B. "Mouse House" and skinner box

Several entrances are created in a burrow when wild or domesticated mice are given the opportunity to dig.[15] Within the constraints of the cage mainly used at NIMR, (the NKP MT1 cage), we felt two entrances to be a sensible maximum. Jumping and climbing are positive natural behaviours.[8] In order to encourage jumping and add a third dimension to the cage, the second entrance was put in the roof. This second entrance is obscured from operator view by the use of an upright flap, which also served as a useful handle, being set in front of it. The mice could therefore exit from the hole without being seen from the front of the cage. We felt that this would encourage the mice to use this entrance more. Having an entrance in a different plane also prevents mice from circling through the insert, or being able to visually monitor both entrances simultaneously.

We hoped that this would prevent the aggression noted in a study where transparent plastic pipes were used.[6] We felt that an entrance in the roof would also act as a vent, allowing heat to escape from the insert. We had previously observed that animals utilising a pipe create a lot of heat, which can cause distress to those in the middle unable to reach either exit (S Clements, NIMR, personal communication). As heat rises, and would be allowed to escape from the insert, we hoped to reduce this phenomenon.

Rodents benefit from a small entrance, through which they can squeeze. Most predators would be larger, so it would be reasonable to assume that rodents feel secure if the entrance is only just large enough for them. The floor level entrance of the insert was designed to be as low as possible; we certainly noticed increased use of the insert as we reduced the height of the floor entrance. It was also long, like an igloo entrance, to resemble the tunnel access to the nest, which also had the benefit of reducing light levels inside the cage insert. The entrance is wider than that of a natural burrow in order to allow pregnant females through. We felt that the insert needed to be able to cope with out-bred laboratory strains capable of having large litters.

Shelves have been shown to be beneficial for rodents.[20] The roof of the insert was kept flat, allowing for an increase in useable floor area being made available to the animals of just over $50cm^2$ ($53.2\ cm^2$). (This equates to floor space for another mouse). This high level space allows for recessive animals to retire from eye contact and possible conflict with more dominant cage mates. It also provides space, perhaps deemed to be desirable territory, for dominant animals to occupy, exerting their dominance without the need for aggression. Space away from the bedding material was deemed to be worth including too, providing an alternative floor material for the mice to rest on. This shelf acts as a roof for animals within the insert, providing overhead protection – recognised as important for animals to feel secure.[20]

To reduce cleaning, there is no base to the insert. It rests directly on the cage bedding substrate. We also wanted to reduce the time taken in removing animals from the insert when cleaning the cage out. When the insert is lifted out of the cage, the occupants remain in the cage, usually within their nest.

The material the insert was constructed from needed to cope with the rigours of regular cleaning in a modern cage washer, and to be able to be regularly sterilised in an autoclave at either 121 or 134°C. The material needed to last as long as modern cage bases, to ensure value for money. This meant that a good quality plastic was the material of choice. The insert in this study was made from polycarbonate which was resistant to chewing or gnawing throughout the study. It is non-toxic to both humans and rodents.

Investigating whether the cage insert was beneficial to laboratory mice

It was felt necessary to prove that the novel insert was beneficial, prior to using it extensively as a standard enrichment provision.

A trial was devised, comparing observable laboratory mouse behaviour in four groups. One group was given no cage insert, one the novel insert described above, and the remaining two were provided with commercially available permanent cage inserts. This was to confirm that cage inserts were beneficial, and compares the different shapes and features to see if one shape scored particularly highly.

The formal mark scheme to score animal behaviour was drawn up to capture three aspects of behaviour. Firstly, positive behaviour traits that we wanted to encourage. Secondly, negative behaviour traits that we felt should be reduced. Thirdly, normal behaviours that we hoped would be unaffected by the inclusion of these inserts.

The positive behaviour traits included:

- Entering and leaving the cage insert, to show that its features were being taken advantage of (not applicable to the control group);
- Resting on the insert. Resting in an awake state is common in wild mice; we hoped that the mice would take advantage of the shelf space and different floor material (not applicable to the control group);
- Self grooming – another common wild mouse activity;
- Sniffing and investigating others – a natural activity which can be achieved in a stable odour cue environment, and which should not result in aggression;
- Grooming others;
- Jumping – a natural activity which is very limited within unenriched cages.

Negative behaviour traits included:

- Prolonged stereotypic bar biting;
- Wheeling from the cage floor to the cage lid, or around in circles on the cage floor;
- Aggressive contact, e.g. biting;
- Aggressive posture, e.g. dominating important areas such as the water sipper;
- Running – indicating a panic response to a supposed predator or a dominant aggressor;
- Sniffing the bedding and the cage – suggesting

concern about their environment, perhaps disturbed and chaotic odour cues;
- Sniffing through the bars – a possible indication that the mice wish to escape.

Thirdly the natural behaviours:
- Sleeping;
- Time awake but stationary;
- Eating;
- Drinking.

Methods

We selected a strain used in large numbers, the BALB/c. It is an inbred strain, ensuring maximum genetic similarity to reduce variability. It is also a strain renowned for fighting and causing husbandry problems when housed in groups.[6] The mice were bred in our separate specific pathogen free (SPF) unit and issued at five weeks of age. They were acclimatised for three weeks in a conventionally barriered experimental animal unit, prior to the start of the trial.

Four separate groups were used. Three groups each had a cage insert, different in each case, one being the novel insert. The fourth group (the control group) had no cage insert added to the cages. Every group consisted of four cages – two cages each containing three female mice, and two cages each containing three male mice.

The novel cage insert, now to be referred to as the "Mouse House", was matched against two commercially available cage inserts. They were selected as the best options for the size of the cage used. We purchased "The Hanger" and "The Eurotunnel" from International Market Supply, at the size appropriate for our cages. They are available in either clear perspex, or perspex in a solid colour. However, as we wanted to ensure that the only variation between the inserts was the shape, replicas constructed in the tinted red polycarbonate that was used for the novel insert the "Mouse House".

The cage used was the MT1 cage, manufactured by North Kent Plastics Ltd. The cage base is made in clear

Figure C(a). Cage with "The Eurotunnel"

Makrolon (BP). We used polycarbonate 250ml water bottles with silicone rings, manufactured by Tecniplast.

The bedding used was Lillico Gold shavings. Nesting was provided by adding tissue paper available as Kimcare medical wipes from Kimberly-Clark, one sheet provided to each cage.

The food was CRM, manufactured by Special Diet Services. Mains tap water was provided *ad libitum.*

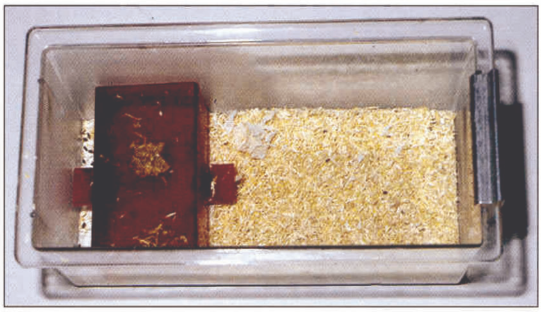

Figure C(b). Cage with "The Hanger"

CAGE ENRICHMENT TRIAL

OBSERVATION CHART

Cage No: 7 Sex: Male

Mouse No: 7

Furniture type: "Mouse House"

Observations	**Date: 24.2.00**		
15 seconds of observation:	1st 5 seconds	2nd 5 seconds	3rd 5 seconds
Stationary/awake			
Asleep			
Eating	O		
Drinking			
Aggressive contact (biting etc.)			
Aggressive posture (non-contact)			
Defensive (being bitten etc.)			
Grooming self			
Grooming others			
Sniffing/investigation of others			
Sniffing bedding			
Sniffing bars			
Sniffing cage sides			
Sniffing through bars			
Gnawing bars		O	
Climbing/holding bars		O	
Floor to bar wheeling			
Base wheeling			
Running around cage			
Jumping			
Climbing on furniture	O		O
Resting on furniture			
Entering furniture			A
Leaving furniture			A
Urination in same corner			
Urination/marking furniture			
Urination/marking cage area			
Droppings			

A = Activity inside furniture

O = Activity outside furniture

Figure D. The Pre-determined activities observation chart

The environmental conditions in the animal room during the trial were a room temperature of 21°C ±2°C and a room humidity of 55%RH ±10%. There were between 15-20 air changes per hour, provided via a large central ceiling plenum and extracted through vents on the side walls behind the cages' racking.

The light cycle was 12 hours light, 12 hours dark, changing at 2am and 2pm. The lights normally change at 7am and 7pm, but the lighting in the animal room used was changed to facilitate observation in work hours. All animals were acclimatised to this timing for three weeks prior to the trial.

The animal room selected had a standard 11.2 m^2 floor area and housed approximately 200 MT1 cages. Breeding lines and stock mice were housed in the room. Scientific users did not access the room and, apart from weekend cover, the same animal technician maintained the room.

We applied and extended existing methodology for the scoring of behaviour,[6] this being an application of "time budget sampling", a technique described by Martin and Bateson.[21] Observations were taken during the night phase of the light cycle, the dark-hours, which is the animals' normal active time. This was made possible by the use of a red light torch, ensuring minimal disturbance. Each animal was observed for five seconds in turn, with an interval to record activity on a pre-set mark scheme. The observations were carried out in the second hour of the dark cycle, daily for five days each week. The study ran for a total of four weeks. The data was obtained by the same animal technician, who ensured that there was very little noise made and that the observations were made without touching the cages, to achieve minimal disturbance.

A separate record book was available and used for comments by the animal technicians responsible for maintaining these cages. These observations were made in the light hours of the light cycle. The observation record book encouraged comments on ease of use of the cage insert, to assess the practicality of the inserts. It also allowed for comments on behaviour of the mice during the light hours, in the animal technicians' own words, and for observations such as the condition of the paper nest when cleaned out, and ease of handling the mice.

At the end of the trial the mice were euthanased by cervical dislocation, and the adrenal glands removed, weighed and compared.

BEHAVIOUR	WHOLE POPULATION COMPARISON	MALES-ONLY COMPARISON	FEMALES-ONLY COMPARISON
Stationary/Awake	No Significant Differences	No Significant Differences	No Significant Differences
Asleep	No Significant Differences	No Significant Differences	No Significant Differences
Eating	No Significant Differences	No Significant Differences	No Significant Differences
Drinking	No Significant Differences	No Significant Differences	No Significant Differences
Aggressive Contact (biting etc)	No Significant Differences	No Significant Differences	No Significant Differences
Aggressive Posture (non contact)	No Significant Differences	No Significant Differences	No Significant Differences
Defensive (being bitten)	No Significant Differences	No Significant Differences	No Significant Differences
Grooming Self	Mh mice groomed significantly more than E, C & H mice	Mh mice groomed significantly more than E mice	Mh mice groomed significantly more than E, C & H mice
Grooming Others	No Significant Differences	No Significant Differences	No Significant Differences
Sniffing/Investigation of Others	Mh mice sniffed/investigated others significantly more than E & C mice	No Significant Differences	Mh mice sniffed/investigated others significantly more than E, H & C mice
Sniffing Bedding	Mh mice sniffed bedding significantly less than C mice	Mh mice sniffed bedding significantly less than C mice	Mh mice sniffed bedding significantly less than C mice
Sniffing Bars	Mh mice sniffed bars significantly less than then E, C & H mice	Mh mice sniffed bars significantly less than then E & C mice	Mh mice sniffed bars significantly less than then E, C & H mice
Sniffing Cage Sides	Mh mice sniffed cage sides significantly less than C & E mice	Mh mice sniffed cage sides significantly less than C mice	Mh mice sniffed cage sides significantly less than C & E mice
Sniffing Through Bars	Mh mice sniffed through bars significantly less than H mice	Mh mice sniffed through bars significantly less than H mice	No Significant Differences
Gnawing Bars	Mh mice gnawed bar significantly more than E & C mice	Mh mice gnawed bar significantly more than E & C mice	Mh mice gnawed bar significantly more than C mice
Climbing/Holding Bars	Mh mice climbed/held bars significantly less than C mice	No Significant Differences	Mh mice climbed/held bars significantly less than C mice
Floor to Bar Wheeling	No Significant Differences	No Significant Differences	No Significant Differences
Base Wheeling	No Significant Differences	No Significant Differences	No Significant Differences
Running Around Cage	Mh mice ran significantly less than E & C mice	Mh mice ran significantly less than E & C mice	Mh mice ran significantly less than E & C mice
Jumping	No Significant Differences	No Significant Differences	No Significant Differences
Climbing on Furniture	No Significant Differences	No Significant Differences	No Significant Differences
Resting on Furniture	Mh mice rested on furniture significantly more than E mice	Mh mice rested on furniture significantly more than E mice	Mh mice rested on furniture significantly more than E mice
Entering Furniture	Mh mice entered furniture significantly more than H & E mice	Mh mice entered furniture significantly more than H & E mice	Mh mice entered furniture significantly more than H & E mice
Leaving Furniture	Mh mice left furniture significantly more than H & E mice	Mh mice left furniture significantly more than H & E mice	Mh mice left furniture significantly more than H & E mice

ANOVA tests performed; post hoc analysis performed using Scheffe test

KEY: Mh = Mousehouse E = Eurotunnel C = Control H = Hanger

Figure E. Statistical findings with significant differences notated

Results

A statistical application was made to the formal data collected in the dark phase of the light cycle. ANOVA tests (Analysis of Variations) were performed, and post-hoc analysis performed using the Scheffe test.

The statistical findings are shown in Figure E with the significant differences highlighted. Statistical significance was determined at a value of less than 0.05.

Positive behaviours

The provision of cage inserts resulted in an increase in certain positive behaviours. Taking the "Mouse House" specifically, there were several positive behavioural traits that had increased significantly.

The mice given the "Mouse House" engaged in self-grooming more than any other group. This was not the frenzied self-grooming seen in distressed animals, but a calm grooming resulting in glossy well kept coats. Wild mice spend long periods of time in this activity. It has been recognised that self grooming may also play an important role in stimulating or releasing body odours.[6]

Female mice in the "Mouse House" engaged in the sniffing and investigation of others significantly more than all other groups, and males more than those in the "Eurotunnel" or control groups. An increased interest in other animals is considered to be a positive result.

"Mouse House" mice entered and left the cage insert more than the other two insert groups. It was encouraging to see that the "Mouse House" was well used. The high level exit was used in addition to the floor entrance, showing that the mice used the third dimension of the insert.

Mice with access to the "Mouse House" were seen to rest on the insert significantly more than the "Eurotunnel" and more than the "Hanger". It seems that any shelf is well used; whether it is because it is higher than ground level or because it is off the bedding is unknown.

A very positive result was the fact that there was continued interest and use of the "Mouse House" throughout the four-week trial. Many enrichment articles require replacing routinely in order to maintain interest.[22] This was not the case in this study. It indicates that the inserts, particularly the "Mouse House", can be kept with the mice continuously. In our experience IVC cages have the potential to go two or three weeks between cage cleaning, so being able to provide enrichment that will be well used throughout this time is considered to be very beneficial.

Negative behaviours

Results showing a significant decrease in negative behaviour traits were also seen when inserts were added.

Instances of mice running around the cage were recorded significantly less in the "Mouse House" than both the "Eurotunnel" and control groups, and less than in the "Hanger" group. Reducing this panic response is considered to be very valuable.

Sniffing through the bars was reduced significantly in "Mouse House" mice compared to those in the "Hanger". This is a positive result, according to Wurbel, who theorises that sniffing through cage bars indicates a desire to escape.[23] It is possible that the "Hanger" is actually providing some stimulation, but there is insufficient complexity to satisfy them. It is very encouraging to note that the mice using the "Mouse House" are obviously able to have their behaviour stimulated, but without seeming to frustrate the mice. This is particularly useful when the cage is contained within an IVC rack, as this style of cage is sealed, so depriving the occupants of external sound and odour cues.

Climbing or holding bars occurred in mice given cage inserts significantly less than the control mice. This indicates that the complexity and three-dimensional aspects of the cage inserts satisfies this particular locomotor activity requirement. It may also indicate that the cage inserts are scent marked, in preference to the bars of the cage lid. Thus contact with the bars to scent and monitor other odours is reduced, and would explain the increase in contact with the inserts. Other studies into the use of shelters in mouse cages have seen similar results.[16]

The action of sniffing bars was significantly less for mice using the "Mouse House" than for all other groups, indicating that the "Mouse House" mice are not scenting and checking odours on the cage lids. Mice in the "Mouse House" sniffed their bedding significantly less than the control mice, and sniffed the cage sides significantly less than control or "Eurotunnel" mice. These results indicate that, since scent marking is a vital feature of mice behaviour, it is the cage insert that has been scent marked. This is beneficial in two ways. Firstly the bedding does not need to be scent marked, which is beneficial as bedding that has been disturbed by mice running across it can result in chaotic odour cues and the increased chance of aggression. Secondly, if the cage insert is moved to a clean cage along with the resident mice, their odour cues go with them. This results in a calm introduction to their new cage without frenzied scent marking or aggression being observed. It is encouraging to note that the "Mouse House" scores particularly well here. It is well known that cage cleaning with no odour cues transferred can lead to aggression[5] but it has also been shown that the transfer of odours can modulate this.[24]

There was one significant increase in negative behaviour recorded in mice held in cages containing the "Mouse House". This was bar gnawing and was significantly more frequent than with mice exposed to the "Eurotunnel" and "Hanger" inserts, though not more than mice in control cages. There may be an explanation for why "Mouse House" mice were observed gnawing bars more than mice in the other two insert groups. Because the mice using the "Mouse House" became truly nocturnal and slept throughout the light phase, they were only active in the dark phase of the light cycle, which was when the data was collected. All the other groups gnawed the bars during the light phase in addition to the dark phase of the light cycle. Interestingly, none of the cage inserts were damaged by gnawing during this study. The fact that mice with inserts all showed reduced bar gnawing, when compared to the controls, indicates that mice held in unenriched cages do perform unnecessary bar bitings.

No aggression was noted throughout the study. This was very encouraging, as BALB/c mice are reported to be more aggressive than many other inbred strains.[6]

Normal behaviours

There were no significant differences between the groups in any of the normal behaviours recorded in the dark phase of the light cycle. This supports the proposal that the "Mouse House" does not adversely affect the science. The time spent eating and drinking was unaffected. This, in addition to the lack of fighting and the suggestion that the mice are less stressed, should all have a positive impact on the quality of science being performed.

The adrenal gland weights of mice exposed to the "Mouse House" were on average lighter when compared to each other group – particularly the male mice. This suggests that the mice were not as stressed, as mice in other groups.[25] Due to one data point being unavailable this can only demonstrate a tendency towards significance, but it does seem to support the observations that the mice appeared calm and easy to handle.

Other observations

In addition to the above findings, there were some positive observations made regarding the "Mouse House" cage insert specifically.

A surprising increase in positive behaviour was observed in the mice with access to the "Mouse House". They had long, undisturbed bouts of sleep during the light phase of the light cycle, with mice sleeping even when animal technicians were working in their room. Mice are naturally nocturnal, so activity during the light phase would normally be considered unusual in wild mice, yet it is common within laboratories. Giving mice the opportunity to retire to an area that allows them to be nocturnal, is considered to be very beneficial.[20]

Another unexpected result was the observation of "Mouse House" mice zoning their cage space to include specific areas used as latrines. This has been reported previously,[7] but had not been observed prior to the trial. The zones seemed to include a faecal latrine and a urine latrine, the other areas being kept clean and dry.

Figure G(a). Cage illustrating zoning

Figure G(b). Cage illustrating zoning

Another positive behavioural observation was that the mice using the "Mouse House" appeared calm and were easy to handle. This allows for an ideal interaction between the animal and the animal technician, making husbandry easy and pleasurable.

The observation of nocturnal behaviour in mice that have access to a dark retreat within a standard cage is considered to be a major improvement in animal welfare. A separate trial was run to assess the specific colour of red that the mice preferred. Interestingly this was a lighter red than expected, which had the additional benefit of allowing excellent visibility for the animal technicians. In this later study, a range of different red "Mouse Houses" were offered to BALB/c mice. The same methodology was used. The behaviour of the mice, and the amount of use made of each insert, were recorded. Although detailed data is not included here, in summary one colour was used significantly more than the others and has been adopted as the colour of choice for commercially available "Mouse Houses". The red colouring needs to act as a "cut-on filter", which only expresses light from a particular wavelength. The quality of the plastic must be good to ensure that there is no light visible in the blue wavelength, which can be a feature of poorer quality plastics.

Conclusions

Cage inserts generally, but the "Mouse House" specifically, resulted in significant increases in several positive behaviours and significant reductions in several negative behaviours. There was no change to normal behaviours such as eating or drinking. There was one significant increase in negative behaviour, bar biting, but a possible explanation is provided.

The data are borne out by the observations of animal technicians, in that the mice use the "Mouse House" well and the mice appear to be calm and content. It was reported that it was practical to use for cage cleaning, cage washing and autoclaving processes.

With the cost of the "Mouse House" kept low, due to the MRC patent arrangements, all the suggested 3 P's of enrichment have been achieved; namely, proof that it works, practicality of use and priced reasonably.

Together with tissue paper as nesting material, the "Mouse House" has become the standard environmental enrichment regime for our mice.

Figure H. Room with "Mouse House" in use

It has also been used successfully with several other mouse strains, including mutant and transgenic mice. It has even been used successfully with Siberian hamsters.[26]

The "Mouse House" conforms to the criterion currently drafted in European legislation, due to come into force in 2003. This requirement states that cage inserts should be designed to allow privacy but also facilitate observation.[2]

Our hope is that this study will encourage more research into proving the benefits of environmental enrichment strategies. Our own studies will continue as we observe mice using the "Mouse House" over a long time course. We also hope to develop and provide a cage insert for laboratory rats.

Footnote.
The use of transparent red in the range mice preferred, and the design features of the "Mouse House" have been protected by the MRC using UK and world-wide patents. Specifically "Improved animal enclosure", filing number PCT/GB01?02949, filing date 03.07.2001 applies.

Acknowledgements

We are indebted to a large number of colleagues, who contributed to this study.

Frances Green, for helping to progress our original ideas and processing the statistical results.

Michael Festing, of MRC Leicester, who advised us on the study design, ensuring we obtained maximal data, using the least number of animals.

Dave Presswell and his team, at NIMR's Mechanical Engineering workshop, who fabricated all the early designs and advised us on the detail of the "Mouse House" from a manufacturing perspective.

David Spillane, of Tecniplast, who arranged for the fabrication (then moulding) of the final design. David was a great encouragement, even at the very early stages when our designs were flimsy cardboard affairs.

The following people helped considerably with discussing the study results and relating it to their knowledge of laboratory and wild mouse behaviour. They are Roger Francis of Bristol University, Charlotte Nevison of Liverpool University, and Penny Hawkins from the RSPCA.

I would also like to thank Jim Bowmaker, of the Institute of Ophthalmology, London, for advice on the types of light that mice can and cannot see. Particularly his advice on how this is measured, together with the pitfalls of "blue spots" in poor quality plastics.

Steve Martin, NIMR, provided spectrophotometry expertise when we were assessing the wavelength of red plastics. He was also able to confirm whether it acted as a cut-on filter, ensuring no other colour was being seen by the mice. This helped us decide on the specific colour red that the mice preferred and confirmed the samples used were pure and not affected by another colour.

References

1 Code of Practice for the Housing and Care of Animals Used in Scientific Procedures. (1989). HMSO ISBN 0 10 210789 0

2 Personal communication with David Anderson, Senior Home Office Inspector

3 Wolfson, S., Lloyd, M. (1998). Handbook of Laboratory Animal Management and Welfare. 2nd Edition. Blackwell Science, Oxford

4 (1994). Animal Procedures Committee Annual Report

5 Poole, T., Dawkins, M.S. (1999). Environmental Enrichment for Vertebrates. In: *The UFAW Handbook on the Care and Management of Laboratory Animals.* 7th Edition. (Poole, T). Blackwell Science, Oxford

6 Nevison, C.M. (1999). Strain-specific Effects of Cage Enrichment in Male Laboratory Mice (*Mus musculus*). *Animal Welfare,* Vol 8, No.4

7 Mortell, N., (2001). Practical Environmental Enrichment for Rats and Mice (The Results of a Survey). *Animal Technology,* Vol 52, No.1, 1-19

8 Buttner, D. (1991). Climbing On the Cage Lid; A Regular Component of Locomotor Activity in the Mouse. *Journal of Experimental Animal Science,* 34, 165-169

9 Spalding, J. (1969). Influence of the Visible Color Spectrum on Activity in Mice. *Laboratory Animal Care.* Vol 19, No.1, 50-54

10 (1998). Housing and Welfare in Laboratory Rats. *Animal Welfare,* Vol 7, 121-136

11 Donnelly, H. (1995). Quality in Laboratory Animals. In: *Laboratory Animals – An Introduction for Experimenters.* 2nd Edition. (Tuffrey, A.A.). John Wiley & Sons Ltd, Chichester

12 Williams, R. (1985). Retina Damage in Pigmented and Albino Rats Exposed to Low Levels of Cyclic Light Following a Single Mydriatic Treatment. *Current Eye Research,* Vol 4, No.2, 97-102

13 Schlingmann (1993).The Animal House: Design, Equipment and Environmental Control.

14 Truszkowski, J. (1974). Utilisation of Nest Boxes by Rodents. *Acta Theriologica,* Vol 19, No.29, 441-452

15 Adams, N. (1981). Mouse (*Mus*) Burrows: Effects of Age, Strain and Domestication. *Animal Learning and Behavior,* Vol 9, No.1, 140-144

16 Birke, L. (1985). Exploratory Behavior of Two Species of Murid Rodents *Acomys cahirinus* and *Mus musculus:* a Comparative Study. *Behavior and Neural Biology,* 43, 143-161

17 Peters, A. (1990). Population Density and Growth Rate in Laboratory Mice. *Laboratory Animals,* Vol 24, 273-279

18 Ward, G. (1991). Environmental Enrichment for Laboratory Mice (*Mus musculus*). *Animal Technology,* Vol 42, No.3, 149-156

19 Jenkins, H. (1998). Sheffield University, IAT Congress poster

20 (1967). Husbandry of Laboratory Animals. Academic Press

21 Martin and Bateson (1986). Measuring Behaviour: An Introductory Guide, CUP

22 Orok-Edom, E., Key, D. (1984). Response of Rats (*Rattus norvegicus*) to Enrichment Objects. *Animal Technology,* Vol 45, No.1, 25-30

23 Wurbel (1996). Stereotypies in Laboratory Mice: Quantitative and Qualitative Description of the Ontogeny of "Wire Gnawing" and "Jumping". In: *Zur:ICR and Zur:ICR NU Ethology,* 102: 371-385

24 Van Loo, P., Modulation of Aggression in Male Mice. *Animal Welfare,* Vol 9, No. 3

25 Manser, C., The Assessment of Stress in Laboratory Animals. RSPCA

26 Personal Communication, University of Trieste