A tunnel is not enough: mice benefit from in-cage provision of a communal shelter as well as a handling tunnel

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Abstract

Mouse shelters can provide mice with security, help them thermoregulate, offer darkness to prevent damage to their eyes and enable climbing and gnawing opportunities. For laboratory mice, there is a vast array of commercially available cage furniture, so choosing which shelter to provide can be difficult. We investigated whether an in-cage handling tunnel alone is sufficient as a shelter or whether an additional cardboard tube or igloo shelter is beneficial. Using 12 cages of adult C57BL/6 mice (5 x female, 7 x male), we conducted a repeated measures experiment, providing the handling tunnel alone or with a cardboard tube or with an amber dome in a randomised order for one week per cage. Cages also contained bedding, nesting material and a gnawing block. We observed that, compared with a handling tube alone, mice with the additional dome sheltered three times more ($P<0.001$), probably because the dome enabled more mice to simultaneously shelter. The dome significantly reduced time spent using nesting material ($P<0.001$), so it may have partially substituted for nesting. Some mice used the cardboard tube less than expected by chance, implying that it reduced the useable space available to mice. When the cage was opened for handling, mice with a dome were less likely to spontaneously enter the handling tunnel ($P<0.001$), so the dome needed briefly removing before tunnel-handling mice. The handling tunnel was not sufficient as a shelter, and a shelter large enough for several mice to occupy it simultaneously should be additionally provided.

Keywords: animal welfare; environmental enrichment; laboratory animals; mice; refinement; shelter

Introduction

Environmental enrichment can increase normalcy and Animal Welfare compared with alternative housing options.[1,2] Whilst environmental enrichment is often envisaged as providing stimulation for animals, some valuable enrichment, such as shelters, can instead provide a safe and peaceful place to rest.[3-5] For example provision of red polycarbonate shelters, either with or without other enrichments, significantly increased the frequency of sleeping in rats compared with other non-shelter enrichments.[6] Consistent with the concept of good sleep quality being important for health, provision of a shelter significantly increased longevity in BALB/cJ males compared with an unenriched cage.[7] However a vast array of shelter types are commercially available for laboratory mice making it difficult to know which type to purchase and whether one is enough.

When offered a choice of nest boxes mice preferred those made from perforated materials rather than solid and with one entrance rather than two,[8] inside the preferred shelters, they were observed resting orientated with their heads towards the single entrance. In another study, mice preferred a cardboard shelter over a red polycarbonate one and almost always brought their nesting material into the cardboard one.[9] Whether through improving rest quality or some other means, shelters can often reduce stress in mice. For example, provision of a cardboard tube significantly reduced bar-biting in ICR mice compared with a barren cage[10] but see Wurbel *et al* (1998) and Nevison *et al* (1999).[11,12]

Providing a peaceful refuge is possibly the most important function of a shelter but – depending on the type of shelter – other functions also exist. For example, some shelters could help mice thermoregulate, because room temperatures comfortable for humans are colder than optimal for mice.[13] Shelters that provide some degree of darkness might help protect mouse eyes from retinal degeneration, in the wild, mice are crepuscular and spend much of their time underground and furthermore many domesticated strains are albino, so mouse eyes are adapted to low light levels and can become damaged if light is inescapable.[14,15] Rats preferred darker shelters over fully transparent ones,[16] so mice may be similar, although Sherwin[17] found no significant preference for transparent versus opaque shelters in mice. Mice are unable to distinguish red wavelengths, which has led to the suggestion that they would perceive transparent red shelters as 'dark' e.g., Bioserv.[18] However during light-phase observations, mice showed a tendency to prefer transparent amber or blue shelters (7/17 mice preferring each colour) over red ones (3/17 mice).[19]

Some shelters can enable, not only resting but active behaviours, such as gnawing and climbing. For example, whilst 4 week old C57BL/6J mice mainly used a box for sleeping, by 8 weeks they shifted towards mainly using the box for active behaviour whilst they slept in nesting material,[20] similarly mice greatly preferred nesting material over tunnels for sleeping in.[17]

Shelters can affect aggression in either direction: for example, reducing it if they help a pursued mouse to flee or if they decrease stress-related aggression; or increasing it if they encourage resource guarding or cause pursued mice to become cornered. Shelters reduced aggression in NIH/S mice compared with nesting material alone with tunnels and nest boxes being equally as effective.[21] On the other hand, provision of a tunnel as a shelter plus nesting material did not significantly affect aggression compared with non-enriched cages but it increased stereotypic bar-biting in some mouse strains which the authors suggested could have been due to stress caused by resource-guarding.[11] Furthermore in male BALB/cAnNCRLBr mice horizontal shelf-like 'shelters' increased fight wounds and corticosterone concentrations and reduced bodyweight gain, suggesting that they increased aggression and stress, whilst nesting material decreased aggression.[12]

Whilst some guidelines for the care of laboratory rodents state that shelters 'should normally be provided', some users are concerned that shelters could increase mouse aggression, or obstruct viewing or handling of mice by staff when health-checking the animals.[4,22,23] In general, the more enrichment there is in a cage, the less visible the mice are.[2] Shelters of certain shapes and materials could be obstructive, as found in rats with opaque shelters with a small entrance hole.[16] However the use of transparent, tinted shelters could enable humans to view mice whilst mice perceive themselves to be hidden e.g. Bioserv (2007), Gjendal *et al* (2018), Datesand (2005).[18,19,24] Provision of a transparent red tunnel to mice along with nesting material led to decreased signs of anxiety in an open field test, and greater weight gain in males compared with only nesting material, without significantly affecting physiological parameters.[25] Provision of a tunnel did not obstruct handling[26] and indeed could help facilitate it as the mice can be lifted within the tube.[27] Use of tunnels as in-cage shelters can facilitate handling, because some mouse strains enter in-cage tunnels more readily than tunnels that are only introduced into the cage when handling the mice.[28]

With the strong recommendation and increasing uptake of lifting mice using tunnels rather than lifting by their tails[27] we started with the premise that handling tunnels should be the minimum in-cage shelter provided for mice.[25,28] However we aimed to investigate whether an additional shelter might benefit mice, if the handling tunnel alone did not enable as many mice to shelter as wanted to shelter or if the tunnel shape or material did not enable certain behaviours. We selected two contrasting additional shelters: a cardboard tube which was opaque and could be gnawed and a transparent amber igloo shelter which had a single entrance and a footprint large enough to accommodate several mice simultaneously with their nesting material.

We hypothesised that, if an additional shelter was beneficial, it should enable more mice to shelter than when it was absent, decrease aggression, increase climbing and gnawing, and it should not obstruct staff from viewing or handling mice. We also predicted that the cardboard tube would be the most likely to be gnawed (a normal mouse behaviour) due to the softer material than the polycarbonate shelters. If the handling tube alone provided insufficient space for resting, the mice would be more active when the sole shelter was the handling tube. Mice would be more likely to build nests within the igloo shelter than the tubes, due to its domed shape and larger footprint. Finally, in terms of potential disadvantages from additional shelters, mice would be more frequently out of sight with two shelters compared with only the handling tunnel and, if the additional shelters were redundant and simply cluttered up the cage, then mice would use them less than expected by chance.

Methods

Animals and housing

In a temperature-controlled room, consistently recorded at 22°C with a mean humidity of 49%, an opportunistic sample of 12 cages of existing C57BL/6 stock mice were used in this study. There were five cages containing 2-4 female mice and seven cages containing 3-4 male mice. The mice were aged 15 weeks +/- 12 days at the start of the study. Routinely, each Tecniplast Blue line 1285L cage (Tecniplast UK Ltd) contained Lignocel bedding (IPS Ltd) with at least a clear polycarbonate handling tunnel (Polycarbonate Clear Mouse Handling Tube, 130mm long x 50mm diameter x 3mm wall thickness, Datesand Ltd), two small aspen chew blocks (small, Datesand Ltd), nesting material (Bed-r'Nest and Nestlets, Datesand Ltd), and food (RM1 diet, Special Diets Services) and water *ad libitum*.

In the month before formal observation of these mice began, each cage was also equipped with a cardboard tube (GLP Fun Tunnel Mini, 76mm long x 38mm diameter, LBS Biotechnology) and an amber polycarbonate dome shelter (Amber Mouse Igloo, 108mm diameter, Datesand Ltd). This ensured that the mice were all accustomed to each item of EE and reduced any novelty effect.

The project was approved by the Royal Veterinary College Level 2 Clinical Research Ethical Review Board (BSc3 201804).

Treatment allocation

The cages were rotated weekly through three different shelter treatments (Figure 1):

1. HT = a colourless polycarbonate handling tunnel;
2. CT = a cardboard tube as well as the handling tunnel; and
3. AD = a transparent amber dome as well as the handling tunnel.

The order of treatment allocation to each cage was randomised using the RANDOM function in Excel, ensuring each cage rotated through all three treatments in a random order. Each cage received every treatment for 1 week, meaning that the data collection ran for 3 weeks in total. The shelters were allocated to the cages at cage-cleaning with the same handling tunnel remaining across weeks.

Behavioural observation

An ethogram was devised on the basis of a pilot study with the purpose of quantifying the behaviour and locations of the mice (Table 1). The behaviours specifically recorded were aggression, bar biting and climbing the shelter. Each one of these behaviours could be directly influenced by the EE in the cage. Other active and inactive behaviour was also recorded as a general catchall, to investigate whether the shelter type influenced activity levels. The ethogram also defined areas of the cage so that the location of the mice could be recorded as a qualifier; importantly, this included whether the mice were in contact with each shelter, so that together with 'climbing shelter', we could determine the extent to which each shelter was used for resting versus climbing.

The location of the nest was also noted at the start of observation, with the locations defined in the ethogram, shown in Table 1, as well as the estimated percentage of each shelter that had been gnawed.

State behaviours were recorded by instantaneous recording every 20 seconds for 2 min per cage, while events took the form of one-zero recording between 20 second intervals.[29] The behaviour and location of each mouse was scan sampled by systematically scanning the cage from front to back, and then left to right, and recorded as the number of mice performing each behaviour in each location.

Observations were recorded four times per week:

(1) Day 1 after the cage had been cleaned at 15:00; (2) Day 3 after cleaning at 11:00; (3) again on Day 3 at 14:00; and (4) Day 7 at 10:00 before the cage was cleaned. Recordings (1), (2) and (4) were made whilst disturbing the mice as little as possible. It was difficult

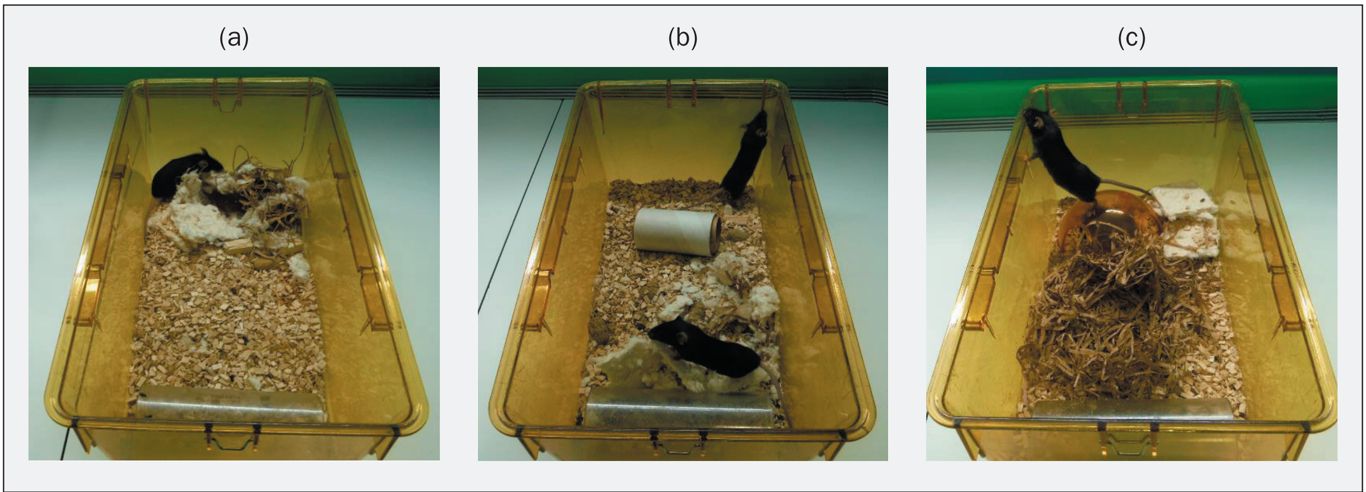

Figure 1. Examples of the three shelter treatments. The photograph in (a) shows provision of a handling tunnel (Treatment HT) only; (b) shows provision of a cardboard tube (CT) as well as a handling tunnel; and (c) shows provision of a transparent amber dome (AD) as well as a handling tunnel. Also visible are aspen gnawing blocks, some scatter-fed food pellets, two nestlets and a Bed-r'Nest (the latter being mostly removed for photographs (a) and (b)).

Behaviour or location	Label	Description	Status
Behaviour	Aggression	Agonistic behaviour such as biting, chasing or boxing.	Event
	Bar-biting	Biting the bars of the cage other than the food hopper.	Event
	Climbing on shelter	Crawling on top of any of the shelters with at least two paws.	Event
	Other Active	Any non-aggressive behaviour or movement, such as walking, grooming, eating, drinking or nest-building.	State
	Other Inactive	Inactive behaviour such as lying, sitting, or standing still.	State
	Out of Sight	Mouse cannot be seen.	State
Location	Handling Tunnel	At least both forelegs inside or on top of the handling tunnel.	State
	Cardboard Tube	At least both forelegs inside or on top of the cardboard tube.	State
	Amber Dome	At least both forelegs inside or on top of the amber dome.	State
	Food hopper	At least both forelegs under or clinging to food hopper.	State
	Open space	Mouse is exposed on the cage floor or cage bars.	State
	Nest	Mouse is inside or on top of the nesting material (if the nest is not within a shelter).	State
	Wall hugging	Mouse's flank is in contact with the cage wall.	State
	Unknown	An observation is missing with unknown location.	State

Table 1. Ethogram of behaviours and locations recorded. At any time, each mouse would be coded with both a behaviour and a location.

to view the cages within the rack, so the cages were gently pulled halfway out from the rack. After 3 min, when the mice had settled again, data were collected without removing the cage or opening the lid. However to assess whether disturbance affected shelter use (because of mice awakening and avoiding potential handling and light) the third observation each week involved removing the cage from the rack and removing the lid under a ventilator hood, as if for a cage-change or handling. The mice were not touched and behaviour observation began as soon as the lid was lifted. This third observation indicated where in the cage the mice regarded as a safe place when their cage was disturbed.

Statistical analysis

Statistical analysis was carried out using IMB SPSS Version 27 (IBM, Portsmouth, UK), with Cage ID as the experimental unit. All observations were divided by the number of mice per cage. The shelter location data were corrected for the amount of floor area per shelter. This was because even if mice had located themselves indiscriminately, they might be observed more at larger shelters simply because of the greater proportion of floor area taken up with the shelters. This correction enabled us to test whether mice spent more or less time using any of the shelter combinations than expected by chance. To calculate the proportion of observations that mice were observed inside, rather than on top of the shelters, the proportion of observations climbing each shelter was subtracted from the total observations of the mice located at that shelter under the assumption that mice could only either be on top of or inside the shelters (see Table 1 for the location and climbing definitions).

General linear mixed models were used for data where the model residuals were sufficiently normally distributed and fitted the model assumptions. If the residuals showed a skewed distribution, the outcome variable was square root or log transformed as appropriate. Data from disturbed mice observed under the ventilator hood were analysed with Treatment, Week, Number of Mice per Cage and Mouse Sex as fixed effects, and Cage ID as the random effect. Data from the three observations per week of relatively undisturbed mice within their racks were analysed similarly but with the Day Since Cage-Cleaning as an additional fixed factor. If any factor showed a significant effect, post-hoc pairwise tests were conducted to examine which treatments differed and in what way.

Where data could not be transformed to fit the assumptions of a parametric analysis, Friedman's or Wilcoxon signed ranks tests were used to compare the treatment effects across the cages. When $P < 0.05$, this was interpreted as being statistically significant.

Results

Shelter use in undisturbed mice

Significantly more mice used shelters in the AD treatment than the other two treatments, both in an absolute sense ($F_{2, 90}$ = 15.42; P < 0.001; Figure 2), and when the floor area taken up by the shelters was taken into account ($F_{2, 90}$ = 90.14; P < 0.001). When the shelter use was separated into climbing versus inside the shelter, climbing showed no significant treatment effect (P = 0.599), but mice were located inside shelters significantly more in the AD treatment than the HT and CT treatments in the absolute sense ($F_{2, 90}$ = ; P < 0.001; Figure 2) and after controlling for the amount of floor space the shelters occupied ($F_{2, 90}$ = 12.92; P < 0.001).

When the amber dome was present, each mouse used it or the handling tunnel on a mean +/- SE of 34.1 +/- 4.0% of the observations, whereas in the HT treatment, they only used the handling tunnel (the only shelter present) on 10.4 +/- 3.9 % of observations; the presence of the cardboard tube reduced shelter use to 7.8 +/- 3.9% of observations. Mice appeared to rarely use the cardboard tube, occasionally climbing the tubes in the CT treatment but hardly ever entering them (Figure 2). Some mice were even observed using shelters less than expected by chance in the CT treatment.

The mice were observed in the shelters significantly less in the absolute sense on Day 7 after cage-cleaning than on Days 1 or 3 ($F_{2, 90}$ = 3.439; P = 0.36). No other effects were found on shelter use.

Home-cage behaviour in undisturbed mice

Mice spent significantly less time interacting with the nesting material in the AD treatment than in the other two treatments ($F_{2, 100}$ = 8.62; P < 0.001). On the other hand, they brought nesting material into the amber dome in 3/12 cages, whereas no mice brought nesting material into either of the tubes. Apart from this, nests were observed in the open floor area on 55% of occasions, under the food hopper on 30% of occasions and against a cage wall on 15% of occasions. Mice interacted with nesting material, including resting in it, more on Day 7 after cage-cleaning than on Days 1 or 3 ($F_{2, 100}$ = 5.20; P = 0.007).

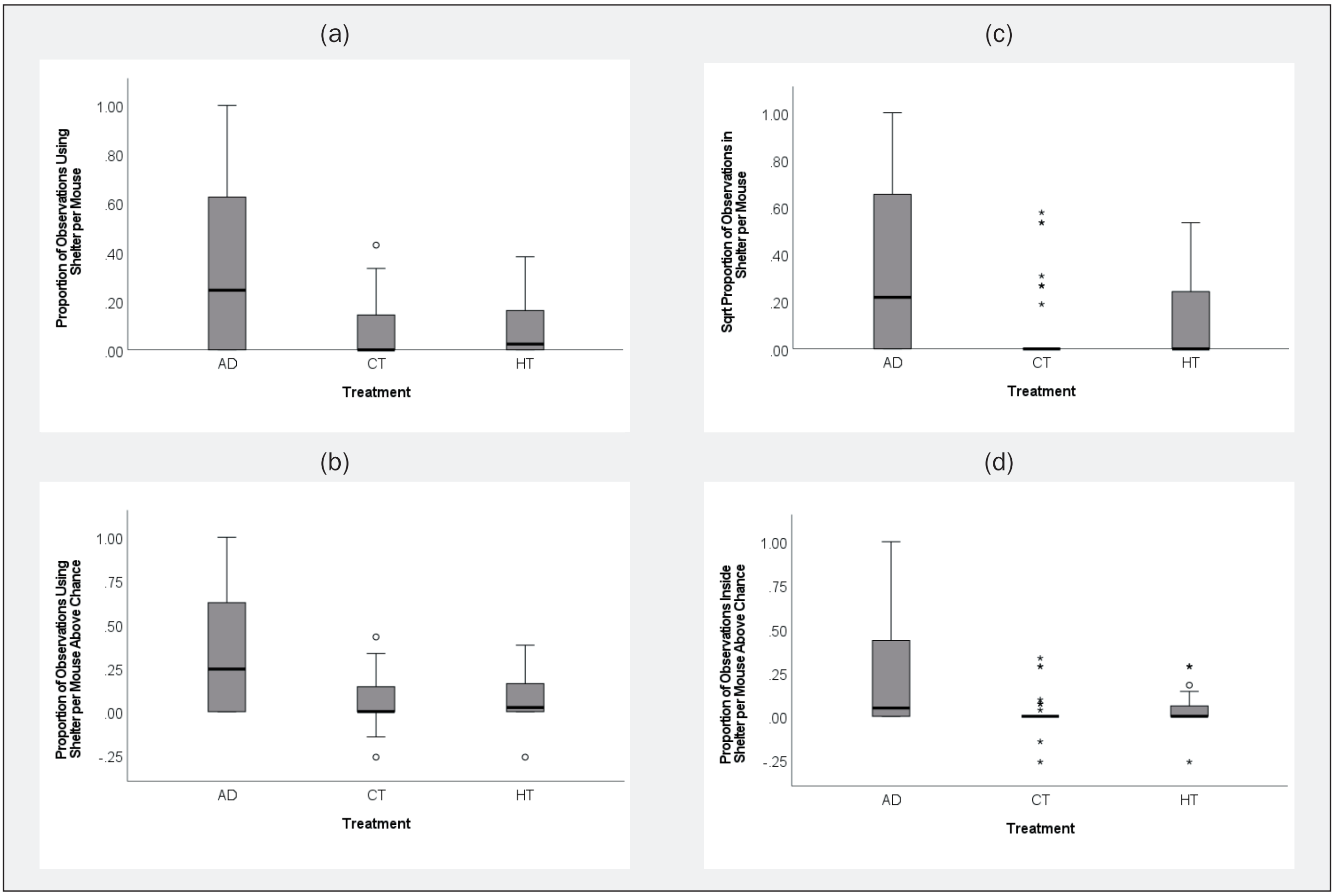

Figure 2. Treatment effects on shelter use in undisturbed mice. The median (+/- IQR) proportion of observations that mice used shelters, including climbing on them, is shown (a) in an absolute sense and (b) relative to the amount of floor space occupied by the shelters. The proportion of observations that mice used shelters, excluding climbing on them, is also shown (c) in an absolute and (d) a relative sense. AD = amber dome plus handling tunnel; CT = cardboard tube plus handling tunnel; HT = handling tunnel only.

Signs of gnawing were seen on cardboard tubes in 8/12 cages, with less than 5% of the tube having been gnawed in each case. Neither of the other shelter types showed signs of gnawing.

Aggression was too rare for analysis being observed in only two cages that contained males (in the AD and HT treatments). Bar-biting was only observed in one cage (in the HT treatment). There were no significant treatment or other effects on activity levels.

Effects on viewing and handling mice

Mice were almost always visible during observations, except for one occasion (in the CT treatment), so it was not possible to statistically analyse for treatment effects on visibility.

Under the disturbed condition in the ventilation hood significantly fewer mice went into the handling tube in the AD treatment than in the HT and CT treatments ($F_{2, 33} = 23.34$; $P < 0.001$; Figure 3). During disturbance, mice were seen inside a shelter on a significantly higher proportion of observations in the CT treatment (58.0 +/- 7.5% of observations) than in the HT and AD treatments (20.6 +/- 7.5% and 35.1 +/- 7.5%, respectively; $F_{2, 29} = 6.39$; $P = 0.005$).

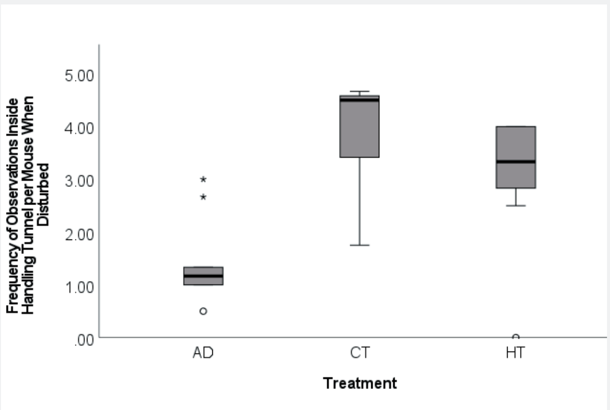

Figure 3. Treatment effects on the frequency of mice entering the handling tunnel when disturbed. The median (+/- IQR) frequency of observations that mice entered the handling tunnel is shown. AD = amber dome plus handling tunnel; CT = cardboard tube plus handling tunnel; HT = handling tunnel only.

Treatment showed no significant effects on activity levels when disturbed but mice were significantly more active when disturbed than undisturbed ($W = 2.94$; $N = 12$; $P = 0.003$).

Discussion

The main results of the current study suggest that C57BL/6 mice of both sexes do benefit from having a communal shelter in addition to an in-cage handling tunnel. Total shelter use during the AD treatment was significantly higher than the other treatments with approximately three times the number of mice using it than when only one or two tunnels were provided (Figure 2). This could suggest that there is insufficient space within the handling tunnel alone for all the mice wishing to shelter to be able to do so. The fact that the provision of the cardboard shelter did little to increase the number of mice sheltering also suggests that the shape, size and possibly the colour of the amber dome also encouraged mice to go inside it. The lack of significant effect of treatment on climbing behaviour suggests that the increased shelter use in the AD treatment was almost entirely because mice were sheltering inside the amber dome not because they were climbing it; the treatment effect on shelter use remained significant even when climbing behaviour was excluded. The presence of the amber shelter significantly reduced time in contact with the nesting material, suggesting that the dome was being used as an alternative to the nest as a place to rest, especially on the first day after cage-cleaning. Mice may prefer to rest huddled together, as they have previously been observed to spend approximately 55% of their time huddled[30] and huddling aids thermoregulation.[31,32]

However if a shelter cannot fit more than one mouse inside, the mice must decide whether to prioritise resting in a shelter or in a huddle, the amber dome might enable them to simultaneously do both, which could enhance welfare.

Mice also brought nesting material inside the amber dome in three of the twelve cages whereas they were never observed to nest in either tunnel type. While mice usually located their nest in other locations around the cage further study could assess whether mice would prefer to have a shelter that can properly accommodate nesting material inside rather than having separate nests and shelters. Visual inspection suggested that even the amber dome was too small to easily accommodate a nest. The mice did not move nesting material into the handling tunnel even though it was seemingly large enough, so perhaps its shape or transparency deterred them from doing so. In future, measuring mouse use of different sizes and colours of dome shelters for nesting could shed light on what shelter characteristics encourage nesting in mice. The amber dome seemed to have attractive qualities for mice in this study but it may be possible to improve the additional shelter still further. For example mice may prefer a shelter made from a perforated material[8] or cardboard.[9] In any case, having a secluded and warm nesting place may be particularly important for breeding mice e.g. Leidinger *et al* (2018), Gaskill *et al* (2013)[33,34] and for mice kept in draughty environments, such as IVCs.[35]

The cardboard tube provided no significant usage above the handling tube alone with some mice seeming to go inside it less than expected by chance when undisturbed. It could be concluded that it was literally a waste of floor space in this study, perhaps being of too narrow a diameter to be useful for adult mice. This finding is perhaps consistent with mice choosing nesting material over tunnels for sleeping in.[17] One potential benefit that the cardboard tube did have for the mice was that it was the only shelter type that showed signs of gnawing, as we hypothesised. The estimated amount chewed in one week never exceeded 5%, so it was in fact chewed fairly little. This could be a finding in favour of the tubes because they remain mostly intact during the course of a week. However, since each cage in this study was already equipped with a wooden gnawing block, the cardboard tubes may have provided little added value even in terms of facilitating gnawing. The mice also did go inside the carboard tube sometimes when disturbed perhaps because its narrowness and darkness made it an attractive refuge from an anticipated human hand.

Some of the potentially negative outcomes that we assessed – aggression, bar-biting and the mice being out of sight – were too rare for statistical analysis. To conclusively test whether the shelter type had any influence on these variables, the length of time for behavioural observation would need to be considerably increased compared with the four 2 minute periods per cage used in this time-limited study. Additionally, they might be might be more sensitively recorded using a continuous or conspicuous behaviour schedule, rather than the one-zero recording used here.[29] Observing the mice in the dark phase under a reversed light schedule would also be beneficial for capturing bar-biting and aggression, as that is when mice are most active e.g. nevison *et al* (2011).[36]

One potentially undesirable logistical effect of the amber dome was that it reduced the frequency that mice spontaneously entered the handling tunnel when the cage was disturbed which could impede capture using the handling tunnel. Therefore, when a shelter is used in conjunction with a handling tunnel, we would advise removing the additional shelter from the cage when mice need to be lifted, thus encouraging the mice to enter the handling tunnel.

For future studies more generally the effect of the number of mice per cage on shelter use should be better understood. For example it would be useful to know the maximum number of mice that would voluntarily rest within a shelter of a given size simultaneously and therefore how many or how large a shelter should be provided for a given group size of mice. It would also be useful to replicate the study with different strains of mice and with breeding mice to assess external validity across mouse strains and reproductive stages.[2,37]

Conclusions and animal welfare implications

Providing mice with a dome shelter as well as the handling tunnel increased the frequency of sheltering behaviour and could thus be a means to increase improve mouse welfare via enhanced security, provision of a darkened area and facilitation of thermoregulation in mice. None of the shelter combinations presented insurmountable logistical difficulties in this study although additional shelters would need to be removed from the cage when mice are due to be lifted via a handling tunnel.

Acknowledgements

This project was funded by a UFAW Animal Welfare Student Scholarship that was awarded to Ria Popat. We would like to thank L. K. Smith and E. Rowley and their team for advising and facilitating on this project and to the researchers who allowed us to observe their off-study mice.

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