



Assessing the safety and suitability of nesting material for singly housed mice with surgically fitted head plates



Zoe Windsor^{a,*}, Simon T. Bate^b

^a University College London, Cruciform Building, Gower Street, London, WC1E 6BT, UK

^b GlaxoSmithKline, GSK Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK

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ABSTRACT

Nesting material, for example shredded paper, is a common form of enrichment for laboratory mice. However, there has been limited research performed regarding its apparent safety when given to mice fitted with exteriorised devices such as head plates. Anecdotally, shredded paper has been deemed unsafe for use with such animals due to frequently observed entanglement. This study assessed the safety of four nesting materials (Pure Comfort White, Rodent Roll, Short Paper Shavings and Facial Tissue) to identify a suitable alternative to shredded paper. The four nesting materials were each tested on 5 head plate mice over a 14-day period. The quality of the nests produced was scored throughout the trial period and incidences of tangling monitored. Tangling was only observed in the Facial Tissue group, and the highest quality nest scores were recorded in the Pure Comfort White group. As shredded paper has been anecdotally alleged to cause entanglement, Pure Comfort White, Rodent Roll and Short Paper Shavings may present more suitable options, given that their use did not result in any incidences of tangling throughout this trial. Pure Comfort White was concluded to be the safest and most suitable nesting material for head plate mice as it produced nests of high quality while reducing the risk of entanglement.

1. Introduction

Wherever animals are used in the laboratory setting, it is our duty to minimise the pain, suffering and distress they experience without compromising the scientific validity of the experimental results. In recent years, much attention has focused on the need to acknowledge, regulate and reduce, where possible, the adverse effects of scientific procedures on laboratory animals, while enhancing and enriching their environment. Environmental enrichment is widely recognised as a method of providing a more structured environment which can improve the well-being of laboratory animals (Baumans, 2005). The provision of an enriched, biologically relevant environment encourages the expression of more species-specific behaviour and allows the animals to control their environment more effectively. Signs of impaired welfare in conventionally housed rodents in barren cages are well documented. A high prevalence of abnormal and stereotypical behaviours, and fear and stress related responses are frequently observed in mice exposed to an environment in which they are unable to perform the natural behaviours required for survival, reproduction and homeostasis (Garner, 2005). Thus, there is resounding evidence that environmental enrichment is a requirement to improve animal welfare (Würbel and Garner, 2007).

Enhancements to the environment not only contribute to better animal welfare, but can also improve the quality of scientific research, as distress in animals can result in physiological changes which potentially increase variability in experimental data, and may even invalidate the research (Garner, 2005; Baumans and Van Loo, 2013).

An easily applicable, and commonly recommended, form of enrichment for laboratory mice is nesting material (Würbel and Garner, 2007; Olsson and Dahlborn, 2002). Materials commonly given to laboratory mice for nest building purposes include hay, straw, clean shredded paper, paper tissues and paper strips. Many studies have highlighted the importance of nesting behaviours to the welfare and daily behavioural repertoire of mice. Additionally, the provision of nesting material has been found to have no detrimental effects on behaviour or physiology, implying mice should be provided with nesting material as a baseline requirement rather than as enrichment option (Hess et al. 2008; Olsson and Dahlborn, 2002).

Nesting material has commonly been studied in relation to pregnancy (Van der Weerd et al., 1997), thermoregulation (Olsson and Dahlborn, 2002 & Gordon, 2004) and welfare (Gaskill et al., 2013a). However, there is limited information in the literature as to its apparent safety. Northrup et al. (2012) found that fibrous nesting material had the

* Corresponding author.

E-mail address: z.windsor@ucl.ac.uk (Z. Windsor).

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potential to become entangled around a mouse's limb, sometimes causing severe injury and resulting in euthanasia. However, further research is required particularly with regard to the use of nesting material with surgically altered animals fitted with exteriorised devices. Such animals often face restrictions on environmental enrichment items such as nesting, as they could potentially become entangled or entrapped, adding to the burden on the animal or resulting in injury. Removing nesting material from the cage is not an appropriate solution, hence adjusting the enrichment protocols is an essential requirement on animal welfare grounds. Furthermore, housing and husbandry conditions that do not accommodate the animals' specific needs may negatively impact research quality (Hawkins, 2014).

The need for a safe nesting material for head plate mice is compounded by single housing. Whilst many facilities make a conscientious effort to group house experimental mice wherever possible, it is still common for mice with head plates to be singly housed when deemed necessary due to scientific or animal welfare reasons. The thermal preference of a singly housed mouse is $\approx 30^{\circ}\text{C}$ (Gordon, 2004), indicating that mice housed under standard laboratory conditions ($\approx 20\text{--}24^{\circ}\text{C}$) must burn additional energy to conserve heat (Gaskill et al., 2011). Failure of homeostasis is a key component of stress (Moberg, 2000), and can compromise many aspects of physiology such as immune function (Yamauchi et al., 1983), pup growth (Gordon, 1993) metabolic parameters (Banet, 1988) and body temperature variability (Yang and Gordon, 1996). Group housed mice have the option to huddle to maintain homeostasis, whereas singly housed mice are more susceptible to thermoregulatory instability at standard housing temperatures (Gordon, 2004 and Gaskill et al., 2013b). Providing mice with suitable nesting material will allow them to alleviate thermal stress by allowing them to control their environment (Gaskill et al., 2011).

In the lead author's own unit mice were routinely given Bed-r'Nest® shredded paper as a nesting material. However, it was observed that mice fitted with head plates can become tangled in this nesting material post-surgery. Head plates are characterised by protruding titanium arms, used to head fix the animal, which the paper strips can easily wrap around. These mice were regularly observed dragging the nest around the cage, unable to free it from the head plate. On three occasions, mice were found rendered completely immobile due to the severity of the entanglement and had to be freed by facility staff. Due to these safety concerns a study was performed to assess the safety of four alternative nesting materials in order to suggest a suitable substitute for use with head plate mice. We hypothesized that the tangling was related to the length and stringiness of the shredded paper nesting, and that materials consisting of shorter fibres would incur less tangling and therefore be a safer option for head plate mice.

2. Materials and methods

2.1. The study subjects

Twenty singly housed mice (10M; 10F) of four genetic strains (Gad2^{tm2} (cre)Zjh./J, N = 12; Camk2a-tTa: emx-cre; Ai93, N = 1; Gad2^{tm2} x Gt (Rosa), N = 4; Som (cre).td.tomato, N = 3) were divided randomly into 4 test groups of 5 animals. The four test groups were: Facial Tissue (2M; 3F), Rodent Roll (2M; 3F), Pure Comfort White (3M; 2F) and Short Paper Shavings (3M; 2F). The age range of the mice was 26–91 weeks at the time of the study (with a mean age of 41 weeks).

Animals assessed in this study were already assigned to experiments that required head-plate surgery. Conducting the study in parallel with ongoing research projects has the ethical advantage of investigating potential refinements and welfare improvements for head plate mice without exposing additional mice to head plate surgery. The quantity, age, strain and sex of the animals was therefore dependent upon the availability of head plated mice already singly housed for experimental purposes within the animal unit.

All subjects were bred to study visual or sensory processing and

visually guided behaviours, and had head plates with glass cranial windows installed to permit optical access to the brain. All subjects were also injected with viruses to introduce two new proteins into their neurons (GCaMP6, an activity sensor, and C1V1, an activity actuator), which are not known to affect mouse behaviour. The mice were singly housed immediately after the virus injection and surgical installation of the head plate and cranial window at approximately 8 weeks of age. Analgesia was provided for 3 days following surgery and no surgical complications were reported.

2.2. Housing and husbandry

All mice were housed in Techniplast™ GM500 IVC cages with ad libitum access to food (2018 Harlan Teklad) and water (Techniplast™ bottle) and lined with Aspen bedding (JRS LIGNOCEL® Wood Fibres). As standard, the mice had been provided with Bed-r'Nest® nesting (The Andersons Lab Bedding), a GLP mini Fun Tunnel (LBS Biotechnology), half a GLP des res dome home (LBS Biotechnology) and a small Aspen Brick (Datesand Group) from birth. All cages were cleaned prior to the start of the study, and were not changed for the duration of the experiment. The animal room housing the mice for this study was an average of 20.6°C and 51% RH. The room maintained a 12:12hr light cycle which began at 07:00.

2.3. Materials

This study assessed the apparent safety of the following four types of nesting material: Pure Comfort White (LBS Biotechnology), Rodent Roll (LBS Biotechnology), Short Paper Shavings (Datesand Group) and Facial Tissue (Office Depot®) (see Fig. 1). Pure Comfort White was selected as it is characterised by small particles which are soft and therefore may be less likely to become tangled on a head plate whilst providing a comfortable material to nest in. It is also absorbent and will clump easily. Rodent Rolls are a variety of compacted nesting material comprised of very short fibres which may be safer for the animal. As the mouse is required to deconstruct and shred the roll, it may also serve as a form of enrichment in more closely mimicking nest building in the natural habitat. Short Paper Shavings were chosen for this trial as they are also characterised by short pieces which may have less potential to become wrapped around a head plate. This material is also soft and comfortable for nest building. Facial tissue was selected for this trial as it is soft and absorbent and is commonly given to mice in labs as a nesting material. Its ability to tear easily may make it a safer option for head plate mice as tangling may be less likely.

2.4. Experimental protocol

Animals were randomly assigned to test groups using the RAND function in Excel, while ensuring that at least two per sex were allocated to each test group.

Approximately 1hr before the dark cycle began, any shreddable environmental enrichment was removed from the cage, leaving only a tunnel and chew stick. The trial nesting material was then added to the rear right quadrant of each cage (5 cages per trial group). The quantity of each material provided was based on perceived amount required to construct a high-quality nest: 5 rolls of Rodent Roll ($\approx 2\text{g}$), 3 sheets of Facial Tissue ($\approx 4\text{g}$), 20g of Pure Comfort White and 20g of Short Paper Shavings.

The visual assessment of nest quality is a subjective process. Prior to conducting the experiment two observers were trained to perform the nest quality assessment. To assess the impact of observer, both observers independently assessed a series of nests during this training. It was found that both scored each nest the same, and hence in the main trial only one observer was employed.

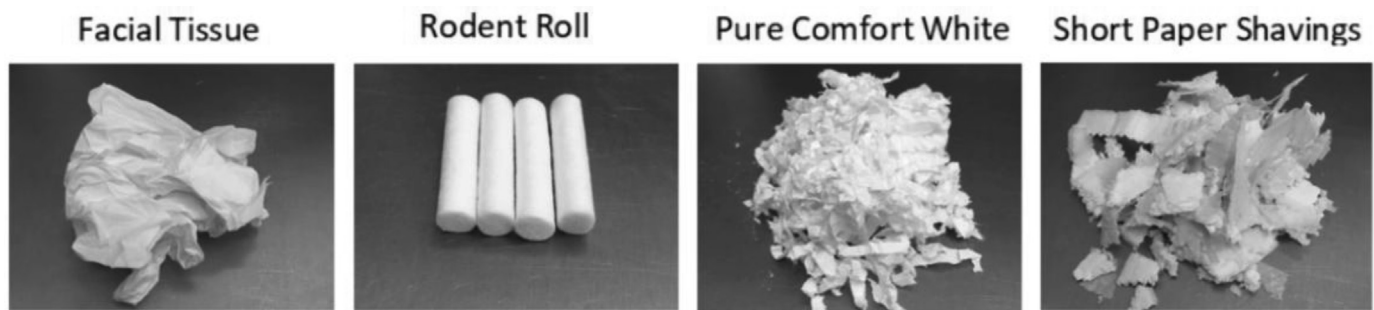


Fig. 1. The nesting materials trialled in this study were selected due to their short fibres and ease of tearing.

2.4.1. Stage 1: assessing the safety of each nesting material

All 20 mice were tested simultaneously over the 14 day observation period.

The observation period began at 8:30am the day after the nesting material was provided to each mouse and lasted for a period of 14 days. During the observational period, the observer recorded *ad libitum* observations about the apparent safety of the nesting material as the mouse interacted with it. At the beginning of each observation period, the mouse was disturbed by being removed from the nest by the base of the tail, as per the current handling practice for head plate mice at the time of the trial. Hurst and West (2010) recommend the use of open hand or tunnel handling in place of base of tail handling to reduce stress and anxiety in laboratory mice. Tunnel handling may not be suitable for head plate mice depending on the type of head plate the mouse has been fitted with, thus open hand handling or cupping may be a more practical refinement. The mouse was scored on ease of removal from the cage without snagging, see Table 1. Observations were made during the early stages on the light phase (which began at 7:00am) as the mice would normally be asleep during this time, and therefore be highly motivated to return to the nest after being disturbed. A prompt return to the nest site would increase the opportunities to record observations about the mouse's interactions with each given nesting material.

Following reintroduction into the cage, for the remainder of the 5 minute observation period animals were scored on the frequency of tangling/snagging observed and, for each tangling/snagging, the severity of the degree of the tangle, see Fig. 2. The observations were conducted by two animal technicians of equal experience (2–3 years) in laboratory animal husbandry. Both technicians held an Institute of Animal Technology Level 2 Diploma in Laboratory Science and Technology, both had completed a BSc (Hons) and one also held an MSc in a relevant animal science.

2.4.2. Stage 2: assessing the ease with which head plate mice can build a nest

On day 1, the undisturbed nests produced in each cage were assessed on a rating scale 1–5 (see Table 2 and Fig. 3) before the mouse was removed from it. The nest quality assessment was repeated on day 7 and day 14.

This study was carried out by the Welfare Group at UCL and falls below the threshold of what is considered a regulated procedure under the Animals (Scientific Procedures) Act 1986. All work was performed under the authorization of one of UCL's Animal Welfare Ethical Review Boards, and at all times the study was undertaken in accordance with the "Code of practice for the housing and care of animals bred, supplied or used for scientific purposes" as published by the Home Office in 2014.

Table 1

Scoring of ease of removal from cage.

Score	Criteria
1	No untangling required-mouse is easily removed
2	Nesting is caught on head plate but easily and quickly removed
3	Intricate untangling of nesting from head plate required

2.5. Statistical analysis

For each animal, the three scores of nest quality, on days 1, 7 and 14, were averaged. The resulting averages were analysed using a 2-way ANOVA approach, with treatment factors Nesting Material and Sex. This was followed by planned comparisons of the predicted means to compare the levels of the Nesting Material factor. The parametric assumptions of the analysis were assessed using the normal probability and residuals vs predicted plots. There was no evidence the assumptions were violated.

As the incidences of tangling was a binary response (i.e. each animal was either tangled or not) then the response was not continuous or normally distributed and hence a parametric analysis could not be performed. As the number of animals in each group was only 5, the incidences of tangling were assessed using the Fisher's exact test.

All statistical results and figures were generated using InVivoStat V3.6, Bate and Clark (2014).

3. Results

3.1. Stage 1: removal from cage

Whilst removing the mouse from the cage, no incidences of nesting becoming entangled on the head plate were observed in any of the test groups.

3.2. Stage 1: incidences of tangling

There was a statistically significant overall difference, across the four groups, in the number of cages where tangling occurred ($p = 0.004$, Fisher's exact test). The only test group in which any incidences of tangling were observed was the Tissue group. There was a total of 10 incidences, in 4 of the 5 cages, observed over the 14-day period ranging in severity from 1 to 3 (5 minor, 2 moderate and 3 severe).

3.3. Stage 2: quality of nests built

As the nests constructed by the mice using Pure Comfort White scored 5 across all three days, the observed data from this group had no variability and was not normally distributed. This group was therefore removed from the dataset prior to the parametric statistical analysis.

During the analysis one animal in the Facial Tissue group, that did not attempt to produce any nests, was excluded as a non-responder (statistically the Studentised residual for this animal was >7). There was no evidence of an effect of sex overall ($F_{(1,7)} = 0.19$, $p=0.676$) but there was a statistically significant difference between the three remaining test groups ($F_{(2,7)} = 5.18$, $p=0.042$), in particular between Rodent Roll and Facial Tissue ($p=0.021$) and Short paper shavings and Facial Tissue ($p=0.036$), see Fig. 4. There was no evidence that the effect of nesting material varied between sexes ($F_{(2,7)} = 0.59$, $p=0.580$).

Additionally, comparing the 95% confidence intervals for the three

Severity Score	Criteria	
Mild	Some nesting material is caught in head plate but mouse can free itself with relative ease	
Moderate	Mouse can free itself but some nesting material still attached to head plate	
Severe	Mouse is unable to free itself; nest is dragged with the mouse when moving around the cage	

Fig. 2. Severity score for each tangle/snagging observed in the observational period.

Table 2

Assessment scale for nesting building phase (adapted from Deacon, 2006).

Score	Criteria
1	Nesting material not noticeably touched (more than 90% left unmoved)
2	Nesting material partially manipulated or shredded (50–90% remaining intact)
3	Nesting material mostly manipulated or shredded but no identifiable nest site (nesting material is torn but the material is not gathered into a nest within one quarter of the area of the cage floor and is spread around the cage)
4	An identifiable but flat nest (nesting material is torn and gathered into a nest within one quarter of the area of the cage floor, but is flat and walls no higher than the height of the mouse's body).
5	A (near) perfect nest (nesting material is torn and gathered into one quarter of the area of the cage floor. The nest is a crater with walls higher than or completely enclosing the mouse)

group means to an absolute score of 5, it is possible to obtain a comparison of the Pure Comfort White group to the other groups. As the 95% confidence intervals for the Short Paper Shavings and Rodent Roll did not include the score of 5, it was concluded that these nesting materials were statistically significantly different from Pure Comfort White.

4. Discussion

4.1. Stage 1: removal from cage

During the observation period, no incidences of nesting material becoming entangled on a head plate as the mouse was being removed from the nest were recorded. This may suggest that all the nesting materials used in the trial are safe, in this regard, and do not pose a risk of tangling whilst lifting the mouse out of the nest.

The mice provided with tissue showed a tendency to grasp onto it upon removal, and therefore frequently dragged the entire nest out of the cage with them. This did not occur with the other nesting materials as the shorter pieces prevented the whole structure of the nest from being disturbed when grasped by the mouse. This is not a safety concern but a convenience consideration for the handler and a potential welfare consideration for the mouse. Daily disturbance of the nest has been shown to have a negative impact on breeding BALB/c mice (Peters et al., 2002), and therefore the possibility of deleterious effect on adult experimental mice should not be discarded. Forcibly removing nesting material from the mouse's grasp also carries the risk of causing injuries such as broken claws.

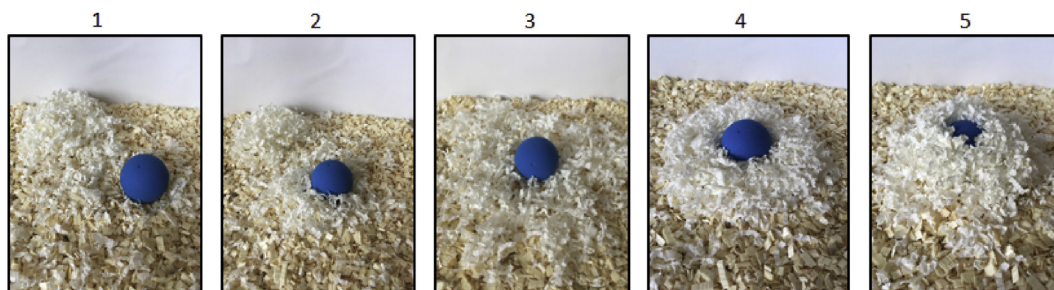


Fig. 3. Example of nests scoring 1–5 using Pure Comfort White (nesting initially placed in rear left of cage for mouse to manipulate). Nests are shown with 45 mm ball for reference.

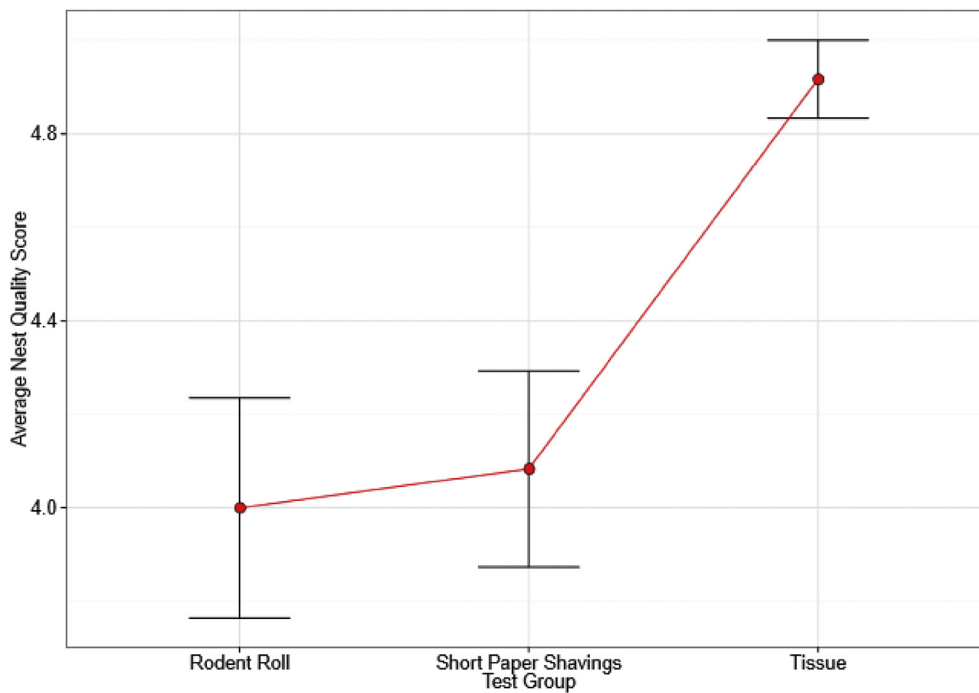


Fig. 4. A statistically significant difference was found between Rodent Roll, Short Paper Shavings and Facial Tissue ($F_{(2,7)} = 5.18, p=0.042$).

4.2. Stage 1: incidences of tangling

Incidences of tangling were only observed in the Facial Tissue group. There was a total of 10 incidences observed over the 14 day period ranging in severity from 1 to 3 (5 Minor, 2 Moderate and 3 Severe). However, Northrup et al. (2012) found that the pitfalls of nesting materials only became apparent during its use on a large scale, as compared to small scale with low numbers of mice. Therefore, tangling may have been observed in the other test groups had the study continued for an increased length of time.

During the trial, it was observed that the Facial Tissue had a tendency to change in structure as the mouse made use of it. The more the tissue was manipulated by the mouse, the stronger and more string-like it became (Fig. 5a & b). Whilst the frequency of entanglement did not increase over time during the trial period, it is possible that this may still have contributed to the incidences of tangling which were recorded in this group. This finding is consistent with studies which suggest that material that is long and fibrous, or can be manipulated into becoming so, poses a risk of injury through entanglement (Rowson and Michaels, 1980; Northrup et al., 2012).

Rowson and Michaels (1980) found that it is important to avoid the use of materials such as cotton wool, as these have a tendency to become entangled around the tails and bodies of young mice and cause injury. Similarly, Northrup et al. (2012) also reported multiple incidences of both neonates and adults becoming caught in fibrous nesting material, with injuries incurred being severe enough to warrant immediate euthanasia. The primary observation in Northrup's study was of nesting material wrapped rigidly around the limbs, severing the blood flow and causing mild to severe cases of reactive oedema and necrosis. One incidence of shredded paper material wound tightly around a mouse's abdomen was also reported.

Thus, certain nesting materials have the potential to become entangled around a mouse's limbs. This potential can easily extend to entanglement around head plates and externalised devices, over which mice have no motor control. This can be comparable to limb paralysis, which renders the animals particularly prone to entanglement in nesting due to the loss of dexterity and manoeuvrability required to free the limb. Guidelines for the care and handling of mice expected to experience hind limb paralysis also advise the provision of a nesting material which can be shredded into small pieces and fibres, such as Nestlets (Dominov; 2011).

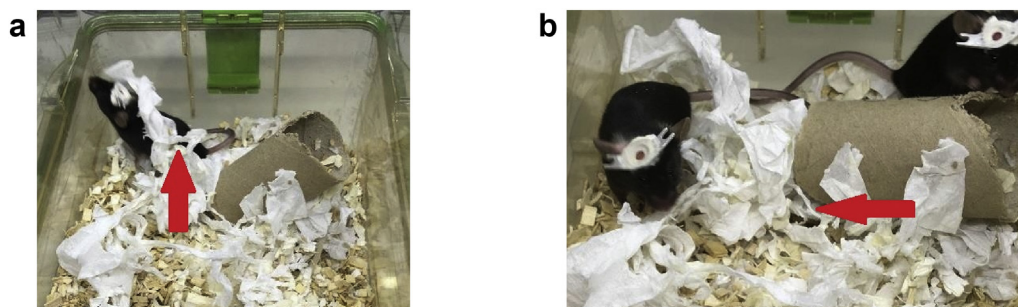


Fig. 5. a & b. The facial tissue displayed a tendency to shred into long string-like strips as the mice manipulated it. These strips also became stronger and no longer tore easily as they did in their original untouched state.

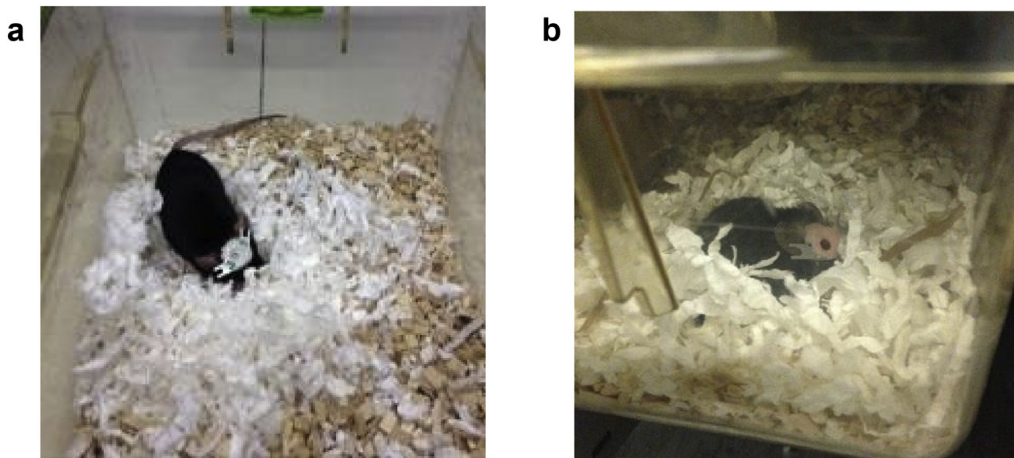


Fig. 6. a & b. Pure Comfort White was used consistently throughout the trial to construct and maintain a near perfect nest.

4.3. Stage 2: quality of nests built

Pure Comfort White was the only trial group to consistently score the highest mark (5) in the nest quality scoring stage, indicating that the mice were consistently able to produce and maintain a near perfect nest throughout the trial (Fig. 6a & b). In the literature, mice have been shown to prefer other paper-based nesting materials over wood-based ones, perhaps due to their ease of shredding and capacity to become more voluminous (Hess et al., 2008). Pure Comfort White also has the added benefit of providing a relatively stable structure through its small compact pieces and capacity for easy clump (LBS, 2017). This structure, combined with the preferred paper constituent, may have been the cause of the consistent high scores it yielded, as the mice were able to build high quality nests with a material that was both structurally sound and seemingly easy to manipulate.

There was a significant difference ($p < 0.05$) between Pure Comfort White (observed score = 5.0) and Short Paper Shavings (average score = 4.3) indicating that Pure Comfort White was used to construct a higher quality of nest throughout the trial. Mice provided with Short Paper Shavings always attempted to gather the material into a nest (Fig. 7a), however much was left behind and remained scattered across the cage, lowering the score of their nest (Fig. 7b). This may be due to 20g of Short

Paper Shavings containing more individual pieces than required by the mouse to build a nest, or it could be that Short Paper Shavings are energetically expensive to carry across the cage into the nest site.

There was a significant difference ($p < 0.05$) between Pure Comfort White (observed score = 5.0) and Rodent Roll (average score = 4.0). Rodent Roll scored the lowest average score of all the test groups, indicating that it is least suitable of the materials tested for producing high quality nests within the 14-day period. An attempt at gathering the rolls into the nest site to form a wall was always made, indicating that the mice did recognise Rodent Rolls as a potential nesting material. However, rolls were frequently found mostly intact and unshredded (Fig. 8). A similar scenario was reported by Hess et al. (2008) when mice were provided with Nestlets. In that study, instead of using the compressed cotton squares to build a nest, the mice simply used them as a border around nests constructed of aspen bedding. This suggests that, despite its lack of use within the nest, certain features of the Nestlet material do still make it attractive to the mice for nest building. Therefore, compressed nesting materials may be preferred for creating nest structure, however in terms of nestability, it does not appear to be favourable. This may be due to material preferences or the energy required to firstly shred the item and then use it to construct a nest.

There was a significant difference ($p=0.014$) between the Facial

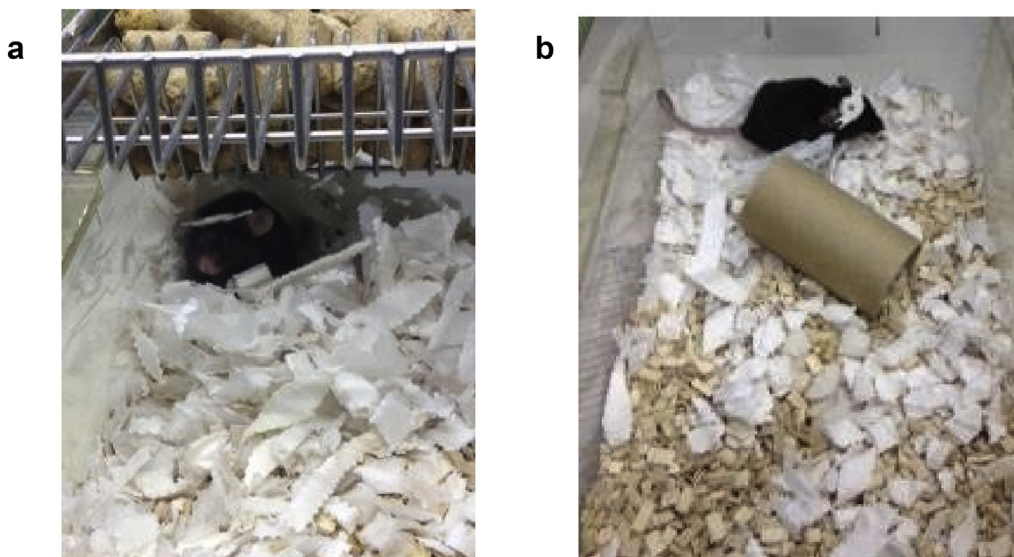


Fig. 7. a. The mice consistently gathered Short Paper Shavings into one nest site throughout the trial. b. Despite the presence of a clearly identifiable nest site, much of the material provided was still left scattered around the cage.

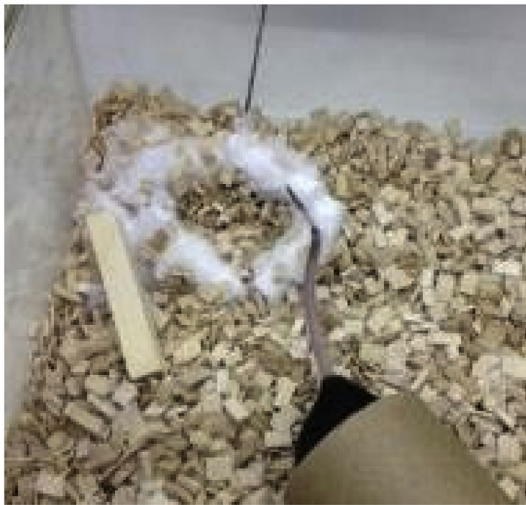


Fig. 8. Rodent Rolls were routinely brought to the nest site and arranged to form a border, however they were frequently left mostly intact and unshredded.

Tissue (average score = 4.9) and Rodent Roll groups (average score = 4.0). Facial Tissue was the second highest scoring test group in the nest quality test as the mice were able to use it to construct and maintain relatively well structured nests throughout the trial. Facial Tissue was initially frequently used to create a nest crater which would completely enclose the mouse, resulting in a high score on the quality scale (Fig. 9). Using a different scoring system Hess et al. (2008) found that mice did not produce high quality nests using only tissues, as compared to with shredded paper strips. However the intermediate score recorded by Hess et al. (2008) is similar in criteria to a score of 4–5 in the scoring system used in this paper (cup shaped nest with walls the height of or higher than the mouse's body), therefore the nests produced in both studies may have been of a similar quality. Hess et al. 2008 also found that when mice were provided with both tissue and shredded paper at the same time, tissue was consistently used to line the nest whilst shredded paper was used to form the structure of the nest. This may indicate that whilst tissue is not ideal for nest building, it still possesses qualities which are somewhat attractive to mice. Van de Weerd et al. (1997) found that C57BL/6T and BALB/c mice show a preference for tissue or towel compared to paper strips and for cotton string wool over wood shavings. The study suggests that whilst a preference for paper derived material is shown, the nature (paper or wood) of the nesting seems to be more important than its structure, as this was directly related to its nestability, or potential quality of the nest produced. Thus, the provision of tissue as a nesting



Fig. 9. Facial Tissue was used to construct nests which scored highly.

material may be beneficial when provided with a second, more naturalistic material, but not as the sole means of constructing a nest. When given the opportunity, laboratory mice have been observed to incorporate multiple materials into their nest (Sherwin, 1997 & Van de Weerd et al., 1997), therefore, providing the opportunity for mice to construct composite nests may be beneficial to animal welfare and a positive refinement to the enrichment protocol.

However in this study, tissue was the only group which yielded incidences of tangling, thus on safety grounds may not be a suitable choice for head plate mice over Rodent Roll, despite having a higher overall nest quality score. Further research into combinations of nesting material which are safe for use with head plate mice is necessary to establish an optimal enrichment regime which contributes to enhanced welfare.

Nest quality in mice is a valuable tool to assess welfare, as the behaviours associated with nest building are highly linked to survival, with wild mice using nests to shelter from predators, harsh environmental conditions and to protect their young (Latham and Mason, 2004). Gaskill et al. (2013a) found that nest building behaviours can alter in laboratory mice depending on thermal stress, and general malaise and pain, indicating that nest building and quality can be used to identify cages suffering reduced well-being. Furthermore, providing mice with a stimulus-rich environment allows the opportunity to engage in rewarding behaviours associated with positive effects such as comfort, pleasure and a sense of control, and therefore, enhanced welfare (Mellor, 2006).

5. Conclusions

5.1. Stage 1: tangling

During Stage 1, no incidences of nesting becoming tangled on a head plate was recorded when the mouse was removed from the nest. This may suggest that all the nesting materials used in the trial are safe in this regard and do not pose a risk of tangling whilst lifting the mouse out of the nest.

The only test group in which any incidences of tangling were observed was the Facial Tissue group possibly due to its long and fibrous nature, thus suggesting tissue is the least suitable of the nesting materials trialled. However, given the short time frame of the study, incidences of tangling may have been observed in other test groups if Stage 1 was continued for longer.

5.2. Stage 2: nest quality

Compressed nesting materials such as Rodent Roll may be preferred by mice for creating nest structure, however in terms of nestability, do not appear to be favourable and may require time to be manipulated into a high quality nest.

In this trial, mice failed to consistently gather all of the Short Paper Shavings provided into a nest site to construct a nest, possibly due to an excess amount given.

Provision of Facial Tissue as a nesting material may be beneficial when provided with a second, more naturalistic material, but not as the sole means of constructing a nest. However, Facial Tissue should not be used for head plate mice due to its potential for tangling.

Pure Comfort White is the safest and more suitable nesting material tested in this trial due to its high nest quality scores and absence of tangling incidences.

This study was constrained by multiple factors. Firstly the sample size was small due to availability of singly housed head plate mice. Limited availability also resulted in the trial mice being of multiple strains and ages. These variables were disregarded for the purposes of this study. Furthermore, the study took place over a short 14 day period. Further research is required into the effects of all these variables on nesting material safety and suitability for head plate mice over a longer study term period. Despite these constraints, the results of this study were

deemed robust enough to implement changes to the local enrichment protocol. All head plate mice in the lead author's own unit have since been provided with Pure Comfort White as standard nesting material, thus the protocol may be useful for other facilities wishing to evaluate nesting materials for head plate mice. Following establishing that a safe nesting material can be provided, further research into mouse preferences in nesting material and combinations of nesting materials is recommended in order to ascertain and provide the best enrichment protocol for head plate mouse welfare.

Declarations

Author contribution statement

Zoe Windsor: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Simon Bate: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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