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Using Paper Nest Pucks to Prevent Barbering in C57BL/6 Mice

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Little research has been conducted to examine the influence of various methods of providing nest materials—such as dispersing them, providing them as single units, or clustering them—on the behavior and welfare of group-housed mice. In this study, 6 wk-old C57BL/6NCrl mice were housed 3 per cage and randomized into 1 of 3 nest-material groups: 1) one facial tissue per cage (control; female mice, 3 cages; male mice, 3 cages); 2) an 8-g 'puck' of compressed nesting material and a facial tissue (females, 3 cages; males, 3 cages); or 3) 8 g of dispersed paper strips and a facial tissue (females, 3 cages; males, 3 cages). Mouse behavior (agonistic, stereotypic, nesting), physical examination data, and nest scores were evaluated over 16 d. The results showed that mice in the puck and control groups spent more time manipulating nest materials after cage changes than did mice in the paper-strip group. Average nest scores were highest in the paper-strip group compared with controls and puck cages. Female cages with pucks showed no barbering, whereas all other female mice cages demonstrated barbering. Overall, nest pucks may provide a time-consuming activity for mice and may help protect female C57BL/6 mice from barbering. However, more research is needed to replicate and expand these study results.

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In a laboratory cage environment, space and resources are limited, and mice do not choose their cagemates. With such limitations and a lack of choice, negative interactions between cagemates often occur, such as aggression and other agonistic behaviors. Agonistic behaviors commonly occur in group-housed laboratory mice,[5,34,35] with consequences such as pain from wounding, stress (subordination or dominance-related), distress (prolonged inability to escape negative interactions resulting in maladaptive effects), and compromised scientific data.[23,34,35] Providing in-cage resources, including adequate nesting material, is important to allow laboratory mice to perform motivated behaviors, to have some control over their environment, and to provide mental and physical stimulation. However, current literature suggests that adding resources such as nest materials can increase,[9,18,26] decrease,[2,3,12,29,32] or have no effect on aggressive behavior in mice.[12,25,36] The interaction of different resources with biologic (that is, strain, age) and external factors (that is, cage environment, noise levels) creates a complex combination of variables that could be different for each cage, room, and laboratory facility.[23,35]

Providing mice with nest material allows them to build high quality nest structures. Various types of nesting materials are available, and the material provided varies across facilities. Nest building is a mouse behavior that is motivated by instinct, and assessment of nest quality could indicate mouse well-being.[14] Past literature suggests that 8 to 10 g of paper-strip nesting material is a sufficient amount of material for mice to build high-quality nests.[14,19] Little research has examined how different methods of providing this material may influence the behavior of group-housed mice. Paper strips can be provided in one area of the cage, dispersed throughout the cage, or provided as a single object, with paper strips woven and compressed into a brown-paper nest 'puck.' How best to provide nest material to group-housed mice is often not discussed. To our knowledge, only one study has examined in-cage placement of mouse resources (tissue, cotton square, sticks, wheel), and the data suggest that clustered resources induce more aggression and stereotypic behavior than dispersed resources.[1] However, that study used non-standard resources and cage set up (large cage environment and atypical resources such as wooden sticks coated in peanut butter),[1] making it difficult to extrapolate the findings to standard laboratory cage systems. The current study investigated the provision of a compact nest material composed of brown paper strips compressed into a puck shape as compared with brown paper strips dispersed in standard laboratory mouse cages. We studied C57BL/6 mice because they are one of the most commonly used mouse strains in research,[15] and mitigating intercage aggression in this strain has important implications regarding improving the welfare of laboratory mice. Male and female mice were housed in same-sex groups of 3 per cage and randomly assigned to one of 3 treatment groups: 1) a single facial tissue (control group); 2) an 8-g 'puck' composed of nesting materials and a single facial tissue; or 3) 8 g of dispersed paper strips and a facial tissue.

Measures used to examine welfare and behavior in group-housed mice included assessing agonistic interactions such as mounting, chasing, biting, resource stealing, and displacement behaviors associated with poor welfare due to subordination stress and distress due to the inability to escape a negative social interaction.[23] We carefully examined mouse interactions with the nest material for comparison with mouse agonistic interactions, given that in-cage resources have been suggested to reduce aggression after cage change.[2] Other parameters examined included assessment of mouse stereotypic behavior (jumping, twirling), because current literature suggests a reduction in stereotypies when mice received high-quality cage enrichment[4,6]

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and improvements in nest quality, which is commonly used as an indicator of mouse welfare.[14,30] Mouse behaviors were examined at the cage level during the dark phase, when mice are most active, as well as after cage change, given that an increase in mouse agonistic interactions has been reported after cage change.[17,33] We predicted that dispersal of paper strips throughout the cage would give all mice equal access to the material and reduce agonistic interactions, as compared with providing nest materials as single-unit items (facial tissue, nest puck) in a single area of the cage. We also predicted that reduced agonistic interactions in cages of mice with the dispersed paper strips would be associated with more time spent manipulating nest materials, less stereotypic behavior, and improved nest quality as compared with providing nest materials as single-unit items (facial tissue, nest puck) in a single area of the cage.

Materials and Methods

Subjects, housing, and handling. Study animals were female (n = 27) and male (n = 27) C57BL/6NCrl mice. The study took place at a Charles River facility in Quebec, Canada, and mice were transferred to the study room at 5 wk of age. Mice were placed into same-sex groups of 3 (n = 18 cages), given that past literature suggests groups of 3 per cage minimizes aggressive interactions[33,34] and for ease of behavioral analyses. Mice were housed in disposable cages (29.5 × 17.7 × 12.7 cm; Innocage, Innovive, San Diego, CA) with static lids (Innocage static MSX2, Innovive), chip bedding (750 mL; Beta-chips, Northeastern Products, Warrensburg, NY) and a single facial tissue (11.4 cm × 21.0 cm; Scott Kimberly-Clark Worldwide, Huntsville, Ontario, Canada), and received food (Charles River Rodent diet 5075, Cargill Animal Nutrition, Minneapolis, MN) and water (Aquavive, San Diego, CA) ad libitum. Mice were kept on a 12:12-h light:dark cycle with lights on at 0800 and were housed for 7 d before the study start. All animal procedures were approved by the Charles River Research Models and Services IACUC.

All mouse handling was performed by using a red transparent tunnel (length, 10 cm; diameter, 5 cm; Bio-Serv, Flemington, NJ) or cupped hands (either or both hands in a cupped position); these techniques were chosen to reduce negative experiences during interactions with researchers.[10,16,21,27]

Experimental testing. On study day 1, cages of mice (n = 18) were blocked by sex and randomized (random number generator) to one of 3 treatments: 1) a single facial tissue (control; females, n = 3; males, n = 3; Figure 1); 2) a facial tissue and a 8-g puck of nesting material (Bed-r'Nest puck, The Andersons, Delphi, IN; females, n = 3; males, n = 3); or 3) a facial tissue and 8 g of dispersed paper strips (Enviro-dri, Shepherd Specialty Paper Watertown, TN; females, n = 3; males, n = 3). For all groups, the tissue was placed in the center of the cage and then a puck was placed centrally (group 2) or paper strips were distributed evenly around the cage (group 3). For consistency, 2 female researchers performed all handling and procedures.

Prior to placement into treatment cages, mice were weighed (in grams; digital scale, AMIR Technology, Shenzhen, China) by using a transparent 100 mL plastic beaker and underwent a physical examination. First, the beaker was slowly tipped sideways onto a handling mat (38.1 × 22.9 × 1.3 cm; VetBed Canada, Smithers, British Columbia, Canada) with a 0.5-cm thick exchangeable fleece top. A timer was started for 15 s, and the mouse could choose to leave the beaker and explore the mat. After 15 s, the mouse's tail was marked (red nontoxic Sharpie, Uline, Pleasant Prairie, WI). A timer was then started for 30 s, during which a physical examination was conducted, including body condition score (score, 1 to 5),[31] coat quality (1, well-groomed, clean, and smooth; 0, dirty or greasy coat, piloerection), and barbering (0, no barbering; 1, missing fur or whiskers or both), and then the mouse was gently stroked multiple times for wound assessment (number recorded). After 30 s, the mouse was placed back into its assigned treatment cage and received a treat (Honey Nut Cheerios, General Mills, Minneapolis, MN, or Fruit Crunchie, Bio-Serv). A small amount of tissue from the previous cage was transferred to the new cage to reduce agonistic interactions.[24,33] Beakers and tunnels were cleaned with 70% ethanol followed by water between mice; gloves and handling mats were changed between cages of mice. Cages were placed on a rack with 3 rows, balanced according

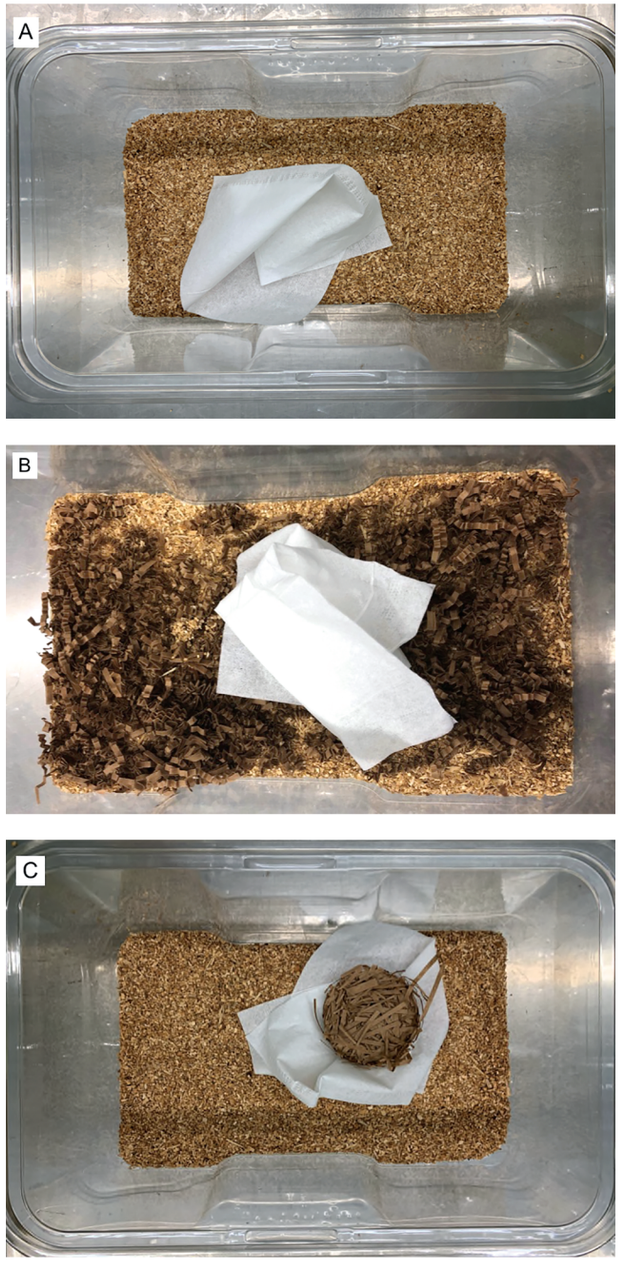

Figure 1. Treatment cages in which mice were housed (3 mice per cage): (A) a single facial tissue only (control; 3 female cages, 3 male cages); (B) a facial tissue and dispersed paper strips (3 female cages, 3 male cages); and (C) a facial tissue and a nest puck (3 female cages, 3 male cages).

to treatment, sex, and row. Cage change occurred on days 8 and 15 by using the same procedure as described earlier. Nest pictures (top and side views; Figure 1) were taken on days 4 and 11. The study was conducted in the home room over 15 d, with a room temperature (mean ± 1 SD) of 21 ± 0.6 °C and relative humidity of 45% ± 5%.

Data collection. Mice were videotaped by using high-definition infrared cameras (1080p Digital Video Recorder 4575, Swann Communications, Santa Fe Springs, CA) for 2-h after cage change and from 1700 to 2100 each day for the duration of the study. Each camera videotaped 2 cages, pointing in the direction of the front of the cages. To ensure high visibility, no cage cards were placed on the cage. Instead, the cages were marked numerically by placing a small sticker in the upper righthand corner of each cage so that the sticker did not block the camera view. Other than the 2 female researchers performing the study procedures, no additional personnel were present in the room throughout the study duration. All video scoring of mouse behavior used Observer XT 14 (Noldus, Wageningen, The Netherlands) software. Mouse behaviors were scored for 2 h after cage change on day 15 and on days 9, 12, and 14 for 4 h (0000 to 0400) during the dark phase. The videos were scored in 15-min segments. Cages were scored in random order using a random number generator with regard to cage number, sex, date, and time interval. Although complete blinding was impossible due to the presence of nest materials in the videos, observers were blind to sex, cage number, and time. Behavioral parameters scored included agonistic behaviors, stereotypic behaviors, material manipulation (Figure 2), and latency to use nest material during the 2 h after cage change (the period from which the cage was placed on the cage rack until one mouse manipulated the materials, or until the 2 h cage-change period was up). For frequency and duration behaviors, a behavioral bout ended when the behavior stopped for at least 2s; if the behavior continued, it was scored as a new bout. A research assistant blind to the study hypothesis scored randomized nest pictures by using a published nest-scoring system.[19] This scoring system was developed to assess nest quality when mice are provided a tissue and paper strip-nest materials. Given that the nest puck is made of paper strips, this scoring system was reasonable to use to assess nest quality for the puck treatment group. However, this scoring system has not been used to assess nest quality when mice are provided a facial tissue only. To make comparisons across treatment groups, we used a single nest scoring system.[19]

Statistical analyses. Analyses were conducted by using SAS Studio version 3.71 (SAS Institute, Cary, NC), with cage as the experimental unit; *P* values less than 0.05 were considered statistically significant. Data are summarized as the mean with the 95% CI provided. Mouse weights were averaged to obtain a mean cage weight on days 1 and 15. Because all mice showed a positive coat quality, body condition score of 3, and no wounds throughout the study, these parameters were not included in analyses.

Stereotypic jumping and twirling data were combined to examine stereotypic behavior. Due to a large number of 0 values for mounting (after cage change, dark-phase scoring) and chasing (after cage change), data were changed to present or absent. Mounting during the dark-phase was analyzed according to a score (range, 0 to 3) based on the sum of the number of 'present' mounting scores over the 3 scoring days. Resource stealing and stereotypic displacement were calculated as rates considering the amount of time that mice were not in view. No biting was observed during the study, and food hopper displacement (after cage change) occurred in one cage; therefore these parameters were not analyzed. Food hopper and stereotypic displacement data were combined to examine the rate of displacement during the dark phase.

The time that mice were out of view was considered when analyzing continuous outcome variables (manipulating materials, stereotypic behavior, chasing, latency to use material postcage change; average dark-phase data) except nest score. All continuous outcome variables including nest score were measured as the logit transformation of time. Continuous outcomes were analyzed by using mixed linear regressions, with cage as the random effect, or multivariate ANOVA with independent variables (treatment, sex, barbering). Model assumptions and fit were assessed by using residual plots, the Anderson–Darling normality test (*P* value less than 0.05), and the adjusted coefficient of determination. Time manipulating materials was nonnormal and thus log-transformed for correction.

The Kruskal–Wallis test was used to examine treatment effects on rate data (number of steals per hour, number of displacements per minute), and the Wilcoxon 2-sample test was used to analyze the effects of sex and barbering on the outcome variables. The Fisher exact test with the Freeman–Halton extension was used to examine treatment effects, and the Fisher exact test was used to measure sex-associated effects on barbering, mounting, chasing, and mount score.

Results

Physical examination. On study day 1, female mice weighed 17.8 g (95% CI, 17.3–18.3 g) on average, and male mice weighed 21.4 g (95% CI, 21.0–21.8) on average. On day 15, female mice weighed an average of 19.6 g (95% CI, 19.1–20.2), and male mice averaged 23.5 g (95% CI, 23.1–24g). All cages of female mice in the control and paper strip groups showed evidence of barbering, whereas no females in the puck cages showed barbering ($P = 0.036$). No barbering was noted in any cage of male mice, regardless of treatment group.

Nest score. Overall, nest scores were higher ($P < 0.001$) in cages of mice in the paper-strip group (mean, 4.4; 95% CI, 3.9–4.9 $F_{2,31} = 36.8$) than in the control group (mean, 2.6; 95% CI, 2.1–3.1) and puck (mean 3.8, 95% CI: 3.4, 4.2; $P = 0.007$) groups. Puck cages had higher ($P < 0.001$) average nest scores than control cages.

Mouse behavior after cage change. The percentage of time spent manipulating nest materials was greater in the control ($F_{3,14} = 8.6$, $P = 0.004$; Figure 3) and puck ($P = 0.03$) groups compared with the paper-strip cages. Cages that did not show chasing spent more time manipulating material (mean, 46.4%; 95% CI, 37%–55.8%) than cages that showed chasing (mean, 33.2%; 95% CI, 24.6–41.8; $P = 0.03$). A greater proportion of female cages showed chasing (odds ratio, 5.5; 95% CI, 1.6–19.3; $P = 0.002$) and mounting (odds ratio, 2.5; 95% CI, 1.2–5.3; $P = 0.0004$) compared with male cages, and a greater proportion of barbered cages showed chasing (odds ratio, 7; 95% CI, 1.1–43; $P = 0.0004$) and mounting (odds ratio, 10, 95% CI, 1.6–64.2; $P = 0.013$) compared with cages without barbered mice.

The number of stereotypic displacements was greater in female cages (median, 19.2 displacements per minute; 95% CI, 11.5–32.5 displacements per minute) than male cages (median, 6.5 displacements per minute; 95% CI, 3.5–9.4 displacements per minute; $P = 0.004$). On average, female cages of mice spent 15.5% (95% CI, 10.2%–20.8%; $F_{1,16} = 12.8$, $P = 0.025$) of their time performing stereotypic behavior compared with 6.4% (95% CI, 2.3%–10.4%) in male cages.

Dark-phase behaviors. Male mice spent more time manipulating materials than did females (odds ratio, 2.6; 95% CI, 1.1–6.1; $F_{1,16} = 6.1$, $P = 0.025$), whereas female mice spent more

Behavior	Operational definition
Agonistic	
Mounting	The duration of time that at least one mouse mounts another mouse in the absence of intromission. Palpations with forepaws and pelvis thrusts may be present
Chasing	The duration of time that at least one mouse chases a fleeing mouse
Biting	The number of times that an aggressor mouse latches onto another mouse by using an open mouth and appears to bite any part of the head, body, or tail
Steal	The number of times that a mouse is seen stealing a resource (that is, bedding, food, feces) from another mouse
Stereotypic displacement	The number of times that one mouse displaces a mouse performing a stereotypic behavior and takes its spot to perform a stereotypic behavior
Food hopper displacement	The number of times that one mouse displaces another mouse to access an area of the food hopper
Stereotypies	
Jumping	The duration of time that at least one mouse spends jumping repeatedly toward the cage top in an upright motion by using the hindlimbs
Twirling	The duration of time that at least one mouse hangs by its forepaws from the cage filter and moves repeatedly in rapid, tight circles in the same direction
Nest material manipulation	
	The duration of time at least one mouse spends manipulating (that is, chewing, dragging, fraying, fluffing) nest material
Out of view	
	All 3 mice are out of view; no behavior can be scored

Figure 2. Ethogram of cage-level mouse behaviors scored after cage change and during the dark phase.

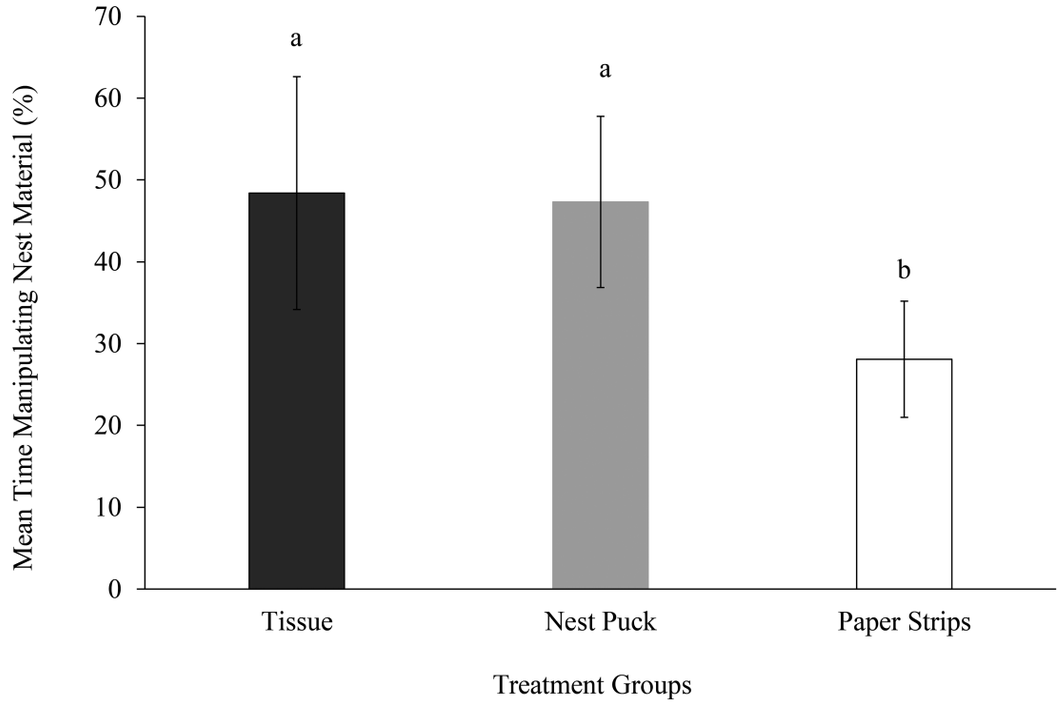

Figure 3. Mean (95% CI) percentage of time spent manipulating nest material by mice housed in control (n = 6), puck (n = 6), and paper-strip (n = 6; n = 18) cages during 2 h after cage change. Different letters indicate $P < 0.005$.

time performing stereotypic behavior (odds ratio; 6.1, 95% CI, 2.9–13.0; $F_{1,16} = 25.5$, $P = 0.0001$) and showed more mounting ($P = 0001$). On average, female mice spent more time performing stereotypic behavior than did male mice (odds ratio, 6.1; 95% CI, 2.9–13.0; $F_{1,16} = 25.5$, $P = 0.0001$).

All other statistical analyses resulted in nonsignificant ($P > 0.05$) findings.

Discussion

In the current study, only female mice in the standard and paper strip groups showed evidence of barbering with missing patches of fur or whiskers, whereas none of the female mice in the puck treatment group showed evidence of barbering. Barbering is a learned behavior that is influenced by many factors, including early-weaning and in-cage resources,[7] a stressful environment,[7,22] and sex; female C57BL/6 mice have shown to be especially disposed to this behavior.[13,28] Past literature suggests that providing high-quality nest material and other resources may reduce the prevalence and severity of barbering in mice.[7] Our results show that cages with barbered mice (female mice in the tissue-only and paper-strip groups) displayed more chasing and mounting than did nonbarbered mice (female puck group and all male groups). The link between barbering and agonistic

behavior is unclear, with some authors suggesting a negative correlation between barbering and aggression in the B6 strain,[22] and others suggesting that more aggression occurs when a dominance hierarchy does not exist in a cage[20] and that a cage with a dominance hierarchy is less likely to show barbering.[11] Thus, no consensus exists. More research is needed to better understand the relationship between barbering and agonistic behavior in C57BL/6 mice.

Although no wounds were detected in the current study, the welfare effects of mild and moderate agonistic interactions are concerning.[23] We saw high amounts of chasing and mounting in barbered cages compared with nonbarbered cages. Chasing and mounting may result in social subordination or dominance stress and is a welfare concern.[8] Sex likely influenced the agonistic behavior (chasing, mounting) and stereotypic behavior seen in female barbered cages. Furthermore, during the dark-phase, female mice showed more chasing, mounting, displacements, and stereotypic behavior compared with male mice, and males spent more time manipulating nest materials than did females. Spending more time spent manipulating nest materials may reduce aggressive interactions. However, more research is needed to elucidate the relationship between nest material use, barbering, and agonistic behavior.

Compared with the nest-puck and tissue-only groups, nest scores were higher for the paper-strip group, suggesting that this material resulted in higher-quality nests. Ideally, nest materials should be suitable for producing high-quality nest structures. Although puck cages showed only moderate nest scores, nest pucks protected mice from barbering, and these mice spent more of their time manipulating nest materials than did the paper-strip group. However, this effect was seen only after cage change and not during dark-phase assessment. Perhaps a mix of paper strips and nest pucks would result in higher nest scores, more manipulation time, and reduced time performing agonistic and abnormal behaviors during the active dark phase. Further research is needed to determine the optimal amount and combination of materials for reducing agonistic and abnormal behaviors and increasing time spent manipulating materials and nest scores.

Limitations of the current study include a small sample size. Also, scoring of the dark-phase recording was not pre-selected based on a 24-h time budget.

The goal of this study was to examine the effects of common nest materials (nest puck, paper strips, facial tissue) on mouse behavior and welfare, including agonistic interactions, abnormal behaviors, and nest material use. To our knowledge, no research to date has examined the use of nest pucks as an effective nest material for improving mouse welfare, although this material is commonly used in research facilities. Our results suggest that nest pucks may be beneficial for group-housed female C57BL/6 mice. Nest pucks consist of condensed, woven brown paper, and mice must unravel the paper to use it. Mice given pucks more manipulation than did mice given paper-strips; consequently, nest pucks may be more mentally and physically stimulating for mice. Although paper-strip cages also were provided a nest tissue, the tissue was placed in the middle of the cage under the paper strips, and mice showed less manipulation of the paper strips and tissue after cage change compared with the other treatment groups. More research is needed to elucidate the relationship between types of nest materials, and manipulation time, barbering, and agonistic behavior. We recommend further research to explore and replicate the benefits of providing nest pucks to group-housed C57BL/6 and other strains of mice and to assess nest materials that reduce agonistic interactions, increase use of nest materials, and result in high-quality nest structures.

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