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Risk factors for barbering in laboratory mice

Anna S. Ratuski[1]✉, Jacob H. Theil[2], Jamie Ahloy-Dallaire[3], Brianna N. Gaskill[5], Kathleen R. Pritchett-Corning[6], Stephen A. Felt[1] & Joseph P. Garner[1,4]✉

Barbering is a common abnormal behavior in laboratory mice, where mice pluck their own fur and/or the fur or whiskers of their cage mates. Barbering mice are a concern for welfare and research quality, as well as serving as a spontaneous model of trichotillomania (a hair-pulling disorder in humans). Causes and prevention of barbering are poorly understood, although there is evidence that both biological and environmental factors play a role in its prevalence. Since initial work in this area was done 20 years ago, mouse husbandry has changed dramatically. We provide an updated analysis of risk factors for barbering in laboratory mice based on point prevalence of hair loss in 2544 cages over one year (7007 mice). We analyzed the effects of biological, environmental, and husbandry factors that are known to be stressors for mice. We found that certain risk factors for barbering, such as sex and breeding status, have persisted despite changes in housing. We additionally identified differences in prevalence based on genetic background, housing system, time of year, and a "hotspot" effect showing spatial clustering of barbering. Our findings can be used to increase understanding of this behavior and to inform changes in husbandry to reduce its prevalence.

Keywords Epidemiology, Stereotypies, Abnormal behavior, Animal welfare, Alopecia, 3Rs

Barbering is a common abnormal behavior in laboratory mouse populations, where mice pluck their own fur and/or the fur or whiskers of their cage mates (Fig. 1). Despite being present in 7–10% of mice[1,2], there are still very few publications on this behavior, potentially due to ongoing mischaracterization of it as a normal part of laboratory mouse behavioral repertoire (e.g., [3,4]). Initial epidemiological work in this area indicated that strain, age, sex, breeding status, being housed with siblings, being housed with a barbering mouse, and use of steel cages were significant risk factors for barbering[1]. These findings were published 20 years ago, and laboratory mouse housing and husbandry has evolved significantly since then. For example, individually ventilated polycarbonate or plastic solid bottomed cages are now commonplace, rather than static plastic or steel cages. A wider variety of bedding materials are now used, and nesting material and/or shelters are more commonly provided[5]. Previous work did not examine seasonal effects which have been identified for other mouse behaviors such as aggression or ulcerative dermatitis[6,7], or spatial clustering of the behavior, (i.e., whether barbers are more likely to be identified in close proximity to other barbers), as has been shown in other species[8].

Only two other studies have attempted to measure risk factors for and prevalence of mouse barbering in the last 20 years. One was a descriptive survey in which respondents from German animal facilities reported perceived prevalence and contributing factors[2], and another was an epidemiological study of 561 cages of mice, replicating some past findings such as higher barbering prevalence in older mice and in females[9]. One commercial vendor has anecdotally described factors that seemed to increase barbering in their mouse colonies: female mice, IVC cages, the winter season, and higher dietary fat[10].

Barbering is performed by both dominant and subordinate mice, as well as singly housed mice[1,11,12]. Causally, barbering is related to elevated oxidative stress[13], and/or stress related to poor environmental conditions[14]. For the recipient, barbering likely leads to impaired thermoregulation due to hair loss[12], impaired sensory perception from missing whiskers[15,16] and pain or discomfort experienced during hair plucking[12,17]. Barbering is often dealt with by separating the barber from other mice, which presents additional concerns for effects on research and welfare of singly housed animals[18,19]. Barbering also parallels the compulsive hair-pulling behavior seen in human trichotillomania in terms of etiology and demographics[12,20,21]. Thus, in addition to the potential welfare benefits of preventing or treating this behavior, barbering mice are a well-established model of human hair-pulling disorders.

[1]Department of Comparative Medicine, Stanford University, Stanford, CA, USA. [2]Campus Veterinary Services, University of California, Davis, CA, USA. [3]Department of Animal Science, Université Laval, Quebec City, QC, Canada. [4]Department of Psychiatry and Behavioral Science, Stanford University, Stanford, CA, USA. [5]Novartis Institute for Biomedical Research, Cambridge, MA, USA. [6]Office of Animal Resources, Faculty of Arts and Sciences, Harvard University, Cambridge, MA, USA. ✉email: aratuski@stanford.edu; jgarner@stanford.edu

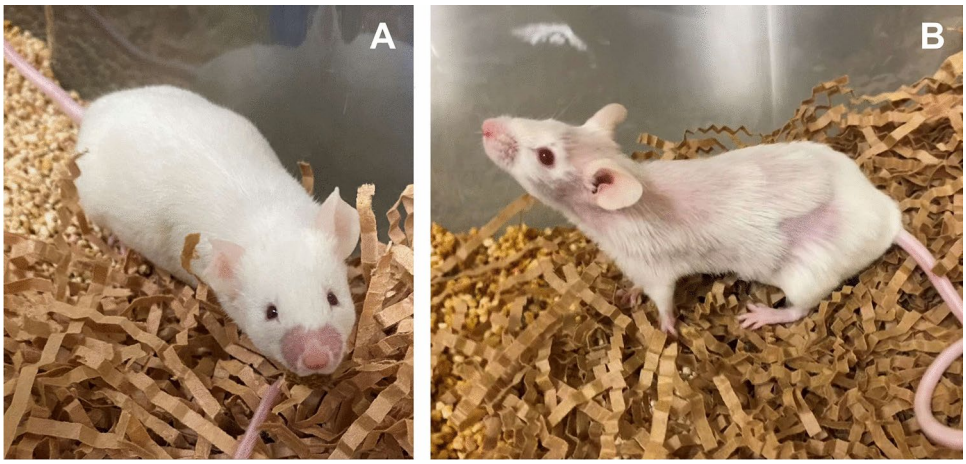

Fig. 1. Examples of (**A**) whisker barbering with some fur barbering around the snout, and (**B**) fur barbering in NSG mice.

To update and expand upon this literature, we used an epidemiological approach to determine prevalence and risk factors for barbering in mice living in contemporary animal facilities across our institution. We analyzed point prevalence of hair loss in 2544 cages (containing 7007 mice total) throughout one year, focusing on biological and environmental factors that are associated with home-cage stress or abnormal behaviors in mice. We hypothesized that factors previously identified as predictors of stress or abnormal behaviors would be associated with a greater prevalence of barbering, such as individually ventilated caging[22,23], corncob bedding [7], lack of shelters[24], breeding cages[20], and cages located higher on the rack[25] or on racks that are oriented parallel to the wall and thus exposed to more disturbance from people[7]. Data-mining and epidemiological studies of this kind cannot capture every variable, but hidden variables may cluster in adjacent cages. As such, we also predicted that there would be "hotspots" of barbering occurring in close physical proximity to other barbering cages, providing evidence of other important factors not included in our measured variables.

Methods

This was an observational epidemiological study of mice housed on the Stanford University campus. Data collection was purely observational and was performed under a protocol approved by Stanford's Institutional Animal Care and Use Committee. The study was performed in accordance with relevant guidelines and regulations and is reported in accordance with the ARRIVE guidelines. All mice were assigned to separate protocols approved by the Stanford Institutional Animal Care and Use Committee. Stanford University is AAALAC accredited.

Experimental design

This serial cross-sectional study sampled point prevalence of barbering in Stanford campus facilities from April 2017 to March 2018. Cage was the experimental unit. Each cage was sampled only once. One veterinarian (JHT) conducted all sampling. In the present study, barbering was the primary outcome, and was identified where fur loss was present on adult mice and the following criteria were met (following[1]): exposed skin was neither reddened nor inflamed; there was no scarring or scabbing; the mouse was otherwise in good health; and there was no other known cause for hair loss. Our sample size was chosen to exceed a target sample from previous epidemiological work in mice[1], as formal a priori sample size calculations do not exist for the types of outcomes and the statistical methods employed here.

Animals

1039 cages contained males, 917 contained females, and 588 were breeding cages. 684 cages were static with woodchip bedding, and 1860 were IVCs with corncob bedding. 1758 cages contained cotton nestlets, 660 contained Enviro-dri paper nesting material, and the rest of the cages contained a combination of the two. 103 cages contained tunnels. 1546 cages were not ear marked, 517 were tagged, and 481 were ear punched. Pair housing was most common, with 847 cages containing two mice. 452 cages contained singly housed mice, and the rest were housed in groups of three to five. C57BL/6 was the most common strain, present in 1136 cages, followed by mixed strains on a C57BL/6 background (553 cages). The remaining strains were categorized as FVB, Swiss background (non-FVB), and other.

Mice were housed in solid-bottom cages with bedding (7092–7097 Teklad corncob bedding or 7090 Teklad sani-chips, Envigo, Madison, WI) and nesting material (6–8 g of crinkle paper, Enviro-dri, Shepherd Specialty Papers, Watertown, TN; 1–2 cotton Nestlets, Ancare, Bellmore, NY; or combination of the two). Individually ventilated caging (IVC) systems were Innovive disposable IVC Rodent Caging Systems (523 cm^2 floorspace, Innovive, San Diego, CA) or Max 75 Plastic Mouse Cages (537 cm2 floorspace, Alternative Design, Siloam Springs, AR). Static cages were Allentown plastic caging (563 cm^2 floorspace, Allentown, Inc., Allentown, NJ) or Innovive disposable caging with static filter tops. Cardboard tunnels (Custom Paper Tubes, Cleveland, OH)

were provided to some mice, for a variety of reasons, by research, veterinary, or animal care staff. Mice were housed in single-sex groups of 1–5 adult mice unless breeding (2–3 adult mice per cage). Health status varied by housing room and included barrier facilities, conventional housing, and biohazard facilities. Our sample included animals bred in-house and sourced from various commercial vendors.

In general, mice were housed in 12:12 L:D light cycles in rooms ranging between 20 °C and 26 °C and 30–70% humidity. Mice were fed ad libitum 2018 Teklad 18% protein rodent diet (Envigo, Madison WI) and provided ad libitum water, unless specifically requested by research or veterinary staff. Innovive cages received Aquavive Mouse Pre-filled Acidified Water Bottles (Innovive, San Diego, CA). All other cages received reverse osmosis treated water in Allentown or Alternative Design water bottles. Any exceptions to normal feed and water were protocol specific and included custom diet formulations and water solutions that were made in-house or purchased from specialty vendors. Cage changes were scheduled once every two weeks for individually ventilated cages (IVC) and once a week for static cages. Nests were generally transferred to clean cages during cage change. Cage racks ranged from 5 to 11 rows and 7 to 8 columns. Manufacturers of racks were the same as the cages they held with the exception of static Innovive caging, which were on Allentown static racks.

Sampling, inclusion, and exclusion criteria

These data were collected at the same time as our previous epidemiological work on male mouse aggression[7]. 2544 cage-level observations (7007 mice total) were included in the final analysis.

Nine central animal facilities were used for sampling (including barrier facilities). Rooms were selected randomly for sampling. Racks and rack sides were chosen randomly within rooms using a dice roll. The starting row on a rack was randomly selected to avoid confounding row with order of observation. For racks perpendicular to the walls of the animal room, sampling began at the column closest to the wall and continued horizontally across the starting row, continuing on lower rows and resuming on the top row until the starting row was reached. For racks parallel to the wall, sampling was conducted from left to right. Any given rack side was sampled only once over the course of the entire study. Rack orientation was recorded at the time of sampling. During sampling, cages were removed from the rack one at a time and visually inspected from outside the cage. Sampling was conducted during daytime. Information from cage cards was collected when available. Each cage was observed once, with an average of 57 cages observed at each time point.

Care was taken to ensure that other factors of interest (e.g., rack orientation), or nuisance variables (e.g., facility) were evenly distributed across time of year. Facilities not managed by Stanford Veterinary Services Center or rooms on reverse lighting schedules were excluded from sampling. Cages with missing data or reasons for missing hair (e.g., nude mice, tumor models, or mice who had been shaven for surgery) were excluded.

Independent variables and methods are the same as those outlined in Theil et al.[7]. Briefly, we recorded sampling date, facility, room, room temperature and humidity, rack number, rack position (parallel or perpendicular to wall or door), in every room sampled. At the cage level, we recorded location on the rack, type of cage (static or individually ventilated), bedding (wood chips or corncob), nesting material (Enviro-dri, cotton nestlets, or both), presence of cardboard tunnels, number of adult mice in the cage, sex, background strain or stock, ear marking method, diet, and signs of aggression.

Statistical analysis

Analysis was performed using JMP Pro 17.0.0 and SAS 9.4 for Windows, and graphs were made using Sigmaplot 14.5. Statistical analysis was planned ahead of data collection, and followed the same approach used by Theil et al.[7]

Data were analyzed using logistic regression, with barbering (at the cage level) being the primary outcome. We followed best practice in model design[26–28], including stress-testing the model for robustness (i.e. "sensitivity analysis")[29]. We first simplified the model with respect to blocking (stratification), rather than hypothesis-testing factors, removing factors producing overspecification as a priority, followed by factors with little variation, or collinearity. These decisions were further informed by AICc (Akaike information criterion, which balances model fit *vs* overspecification). Analysis was initially blocked by building. However, building was not significant, and produced model solutions with significantly worse AICc values. Building was therefore excluded from further models. Other factors with little variability (e.g. diet, temperature, humidity) were removed from analysis. Finally, to avoid overspecification of the model, bedding and cage ventilation were combined for analysis due to collinearity when assessed separately. Similarly, strains or stocks were sorted into larger categories during data processing by an author who was blinded to research outcomes.

In order to capture the influence of effects which were excluded, or unmeasured, an independent hotspot effect (i.e., total number of adjacent cages within two rows or columns containing a barber) was calculated by first taking the logit of an adjusted proportion (adding 1 to all values to eliminate zeroes; and then using a general linear model controlling for all factors included in the logistic regression to calculate a residual. Expressing the hotspot effect as a residual allows an assessment of the hotspot effect independently from all other factors in the final logistic regression model, effectively accounting for variability that is not explained by the predictors we measured. Accordingly, we calculated this hotspot effect with or without correction for building, for use in logistic regressions that did or did not include building.

Factors in the final model included number of mice, presence of shelter, type of nesting material, genetic strain, date, cage ventilation and bedding type, rack adjacency to the wall, cage row on the rack, ear marking method, hotspot effect, sex, and breeding status. Date was modelled as a quadratic function, and tested with a custom joint test of the linear and quadratic terms. The effect of breeding was tested hierarchically, with sex nested within breeding status. The final model was chosen based on lowest AICc values. Post-hoc Tukey tests were performed for genetic background.

Results

Overall, barbering was present in 192 out of 2544 cages (prevalence of approximately 7.5%). Risk factors are presented in order of influence on the model (LogWorth). Number of mice in the cage significantly predicted barbering (LR $\chi^2 = 40.24$, DF = 1, P < 0.0001; Fig. 2A), with more barbering observed in cages with more mice. Hotspots (adjacency to barbers) also predicted barbering (LR $\chi^2 = 36.84$, DF = 1, P < 0.0001; Fig. 2B), as did the presence of a cardboard tunnel in the cage (LR $\chi^2 = 17.57$, DF = 1, P < 0.0001; Fig. 2C). Breeding status significantly predicted barbering, with barbering more likely in breeding cages (LR $\chi^2 = 14.81$, DF = 1, P = 0.0001; Fig. 2D). Strain also predicted barbering, with barbering being most likely in mice bred on a C57BL/6 mixed background (LR $\chi^2 = 17.73$, DF = 4, P = 0.0014; Fig. 2E). Time of year significantly predicted barbering (quadratic LR $\chi^2 = 14.90$, DF = 2, P = 0.0006; Fig. 2F). Barbering was also predicted by housing system, with more barbering seen in mice housed in ventilated cages with corncob bedding (LR $\chi^2 = 7.10$, DF = 1, P = 0.0077; Fig. 2G), and sex (in stock cages), with more barbering seen in females (LR $\chi^2 = 3.89$, DF = 1, P = 0.0485; Fig. 2H).

Position of the cage on the rack (LR $\chi^2 = 0.06$, DF = 1, P = 0.8013; Fig. 3A), position of the rack in the room (LR $\chi^2 = 1.52$, DF = 1, P = 0.2171; Fig. 3B), ear marking method (LR $\chi^2 = 1.53$, DF = 2, P = 0.4660; Fig. 3C), and type of nesting material (LR $\chi^2 = 4.71$, DF = 2, P = 0.0950; Fig. 3D) all failed to predict barbering.

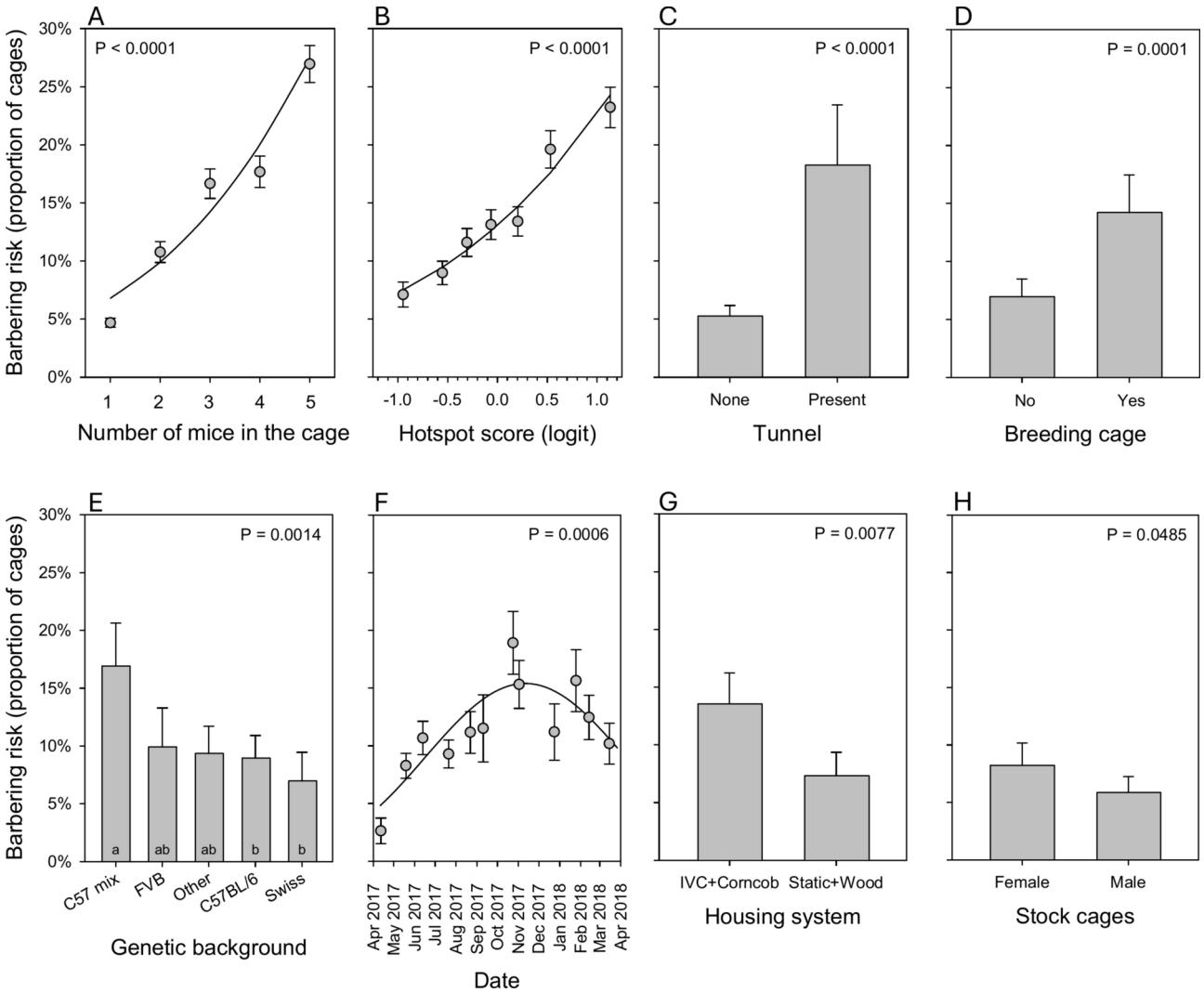

Fig. 2. Risk factors that significantly predict barbering: (**A**) number of mice in the cage, (**B**) hotspot score (adjacency to other barbering cages), (**C**) provision of cardboard tunnels, (**D**) breeding status, (**E**) genetic background (where "C57 mix" refers to mixed strains on a C57BL/6 background), (**F**) date of observation, (**G**) housing system (as a combination of cage type and bedding), (**H**) sex (in single-sex stock cages). For continuous predictors, an equivalent LSL (least-squares line) was calculated at the mean value of all other effects in the model, then for each data point a residual was calculated and added to the LSL. For clarity of presentation, these data were then binned, and the averages of these corrected observed values are plotted within each bin along the x axis, with error bars showing SE. For categorical outcomes, least-squares mean and SE are shown. Letters within bars of panel E indicate Tukey post-hoc tests, where bars with the same letter do not differ significantly. N = 2544 cages.

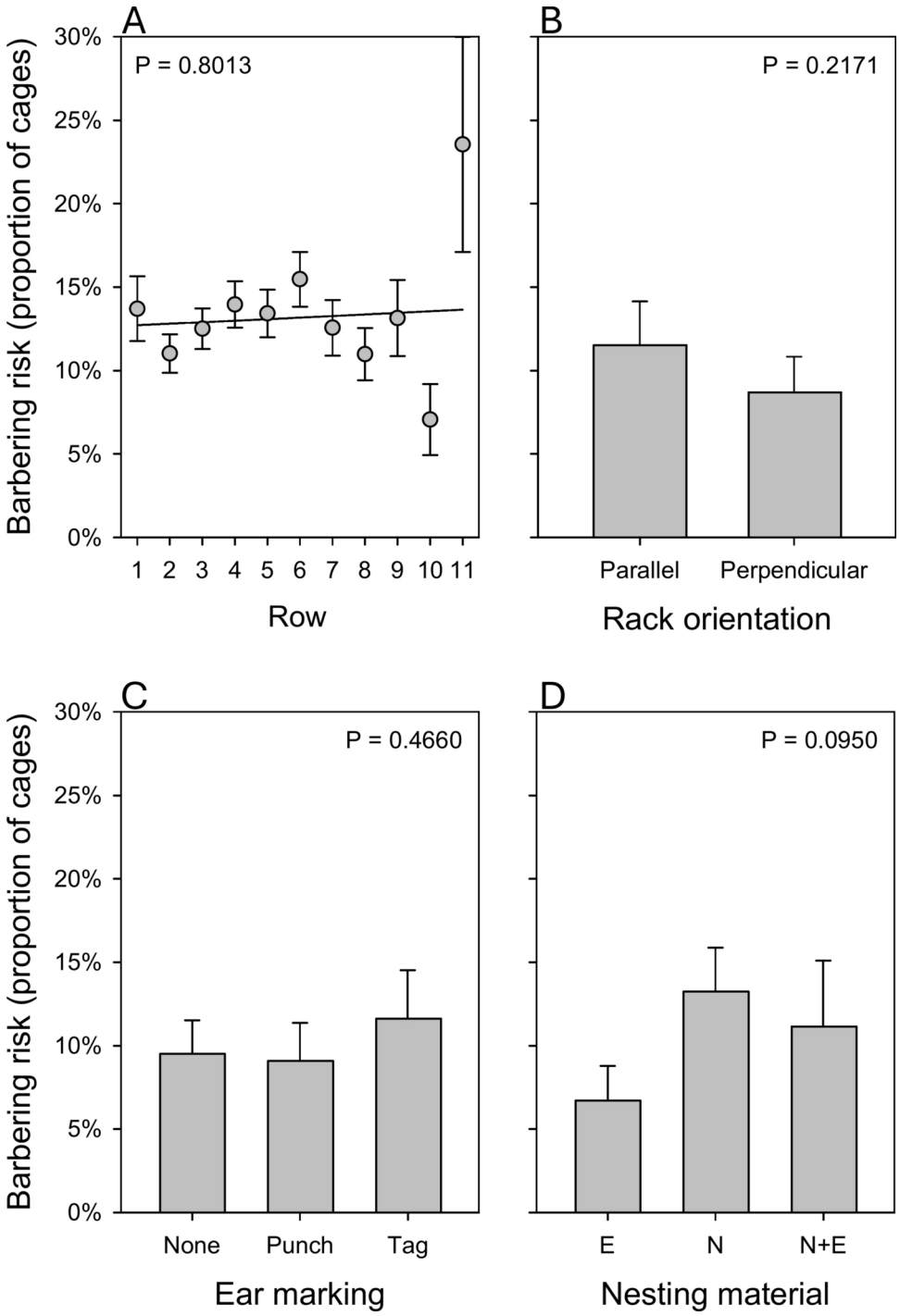

Fig. 3. Factors that did not predict barbering: (**A**) position of the rack in the room (where row 1 represents the top of the rack), (**B**) orientation of the cage rack, (**C**) ear marking method, D) type of nesting material (where *E* Enviro-dri, *N* cotton nestlet, *N* + *E* both). N = 2544 cages.

Discussion

Our study demonstrates that certain risk factors for barbering have persisted despite changes in housing and husbandry in recent decades: breeding, with more barbering seen in breeding cages; and sex, with more barbering seen in females. Genetic background has also remained a persistent risk factor, but our study provides new insight into strain effects, with more barbering seen in mixed strains bred on a C57BL/6 background (i.e., transgenic or congenic mice, often with C57BL/6 and 129 mixed backgrounds). We identified several new factors affecting prevalence of barbering in modern animal research facilities: housing system and bedding type, with more barbering seen in mice housed in ventilated cages with corncob bedding; being housed in close proximity

to other barbering mice; time of year, with an increased frequency in the fall; and number of mice in the cage, with more barbering seen in cages containing a higher number of mice.

As expected, barbering was more likely to be seen in breeders and in female mice. This provides further evidence of a biological basis for the sex bias seen in human trichotillomania patients (i.e., higher levels of trichotillomania in women are not simply due to differences in reporting or diagnosis between male and female patients, as has been suggested more recently[30]). Previously, female mice were identified as 1.5 times more likely to be barbers than males, and breeders were approximately five times more likely to barber than mice housed in single-sex cages[20]. One recent experiment found barbering in female mice only[31]. In human trichotillomania patients, hair pulling is more common in female patients and tends to start during development of secondary sex characteristics[30,32]. In females and reproductive individuals, this could be linked with oxidative stress[33]. Treatment with N-acetylcysteine (a precursor to the synthesis of glutathione, which is a defender against oxidative stress[34]) is effective at reducing trichotillomania or compulsive behavior in human patients[35,36]. Similarly, treatment of female mice with N-acetylcysteine prevents and reduces barbering[13]. This highlights the role of oxidative stress in the occurrence of this behavior, with the clear potential for sex differences related to sexual development and reproductive status.

Barbering was most prevalent in mice of mixed strains bred on a C57BL/6 background. Reported strain effects have been mixed[12], with C57BL/6 mice frequently regarded as having a high-barbering phenotype[2,4,10,37] although there are reports of barbering being relatively absent in this strain and highest in mice on 129SvE backgrounds[38]. C57BL/6 mice are often expected to barber by default, even by some commercial mouse vendors[4,10]. Our data do not support the notion that C57BL/6 mice are a high-barbering strain compared to other strains. Mixed strains appear to be at a worse risk for barbering when C57BL/6 genetics are crossed with other strains that are at high risk of barbering, such as 129 mice[12,38,39].

IVC cages with corncob bedding led to a higher likelihood of barbering, which mirrors the effects found on mouse aggression[7]. The analysis of these variables highlights challenges that exist in analyzing epidemiological data. First, we can only test the effects of factors that vary in the population. Second, some factors may be sufficiently collinear that the analysis will produce a false negative if both are included, in which case they had to be combined (e.g., cage ventilation and bedding in this case), or excluded (e.g., building in this case) based on biological reasoning, simplifying the model, or optimizing model fit. Impacts of IVCs and corncob bedding are discussed in more detail in Theil et al.[7]. Briefly, IVC systems negatively impact mouse welfare, causing increased anxiety behavior[23], cold stress[22], and cage rack vibration[40]. Corncob bedding additionally raises several red flags for rodent welfare, including increased aggression[7,41], altered reproductive behavior[42], hormone disruption[43], and the fact that rodents prefer wood-based bedding over corncob[44]. Estrogen contributes to masculinization of the brain before birth and development of sexually dimorphic behavior during puberty[45]. Corncob contains compounds that suppress aromatase (which converts androgen to estrogen) and disrupt estrogen signaling[42]. Thus, being raised on corncob disrupts estrogen signaling in male animals[46]. Male aromatase knockout mice display increased barbering and other female behaviors, demonstrating the effects of estrogen suppression on behavioral development[47], consistent with the effects of corncob seen here.

Speculatively, it is possible that environmental variables influence the risk of barbering endogenously by modulating oxidative stress through HPA axis activation[48] or increased metabolic stress. For example, cold stress related to cage temperature and ventilation has been linked with higher metabolic demands in mice, especially when appropriate shelter or nesting material is not provided[22,49], which could increase oxidative stress and risk of barbering through increased metabolic stress. Additionally, mouse diets are not homogenous and may not be formulated with appropriate nutritional content[50]. An imbalanced diet lacking in amino acids or other compounds related to antioxidant capacity could also contribute to oxidative stress[34,51] and observed barbering levels. The relationship between these variables and mouse responses to glutathione precursors such as N-acetylcysteine could be tested more directly in future experiments.

We identified "hotspots" of barbering, where cages containing barbers were more likely to be spatially clustered within two cages of other barbers. Just as being housed in the same cage increases likelihood of barbering[1], mice housed in close proximity to another barber have a higher likelihood of barbering. Social transmission of abnormal behavior between neighboring animals has been suggested in experiments with bank voles[52] and pigs[53]. However, social transmission should not be assumed until all environmental hotspot effects have been ruled out. For example, in parrots performing feather-plucking behavior, spatial clustering of the behavior within the housing room could be explained by a combination of other factors such as genetic relatedness, rather than through social transmission[8]. Because mice have limited visual ability[54] with limited access to other cages (especially those in rows above and below) and are contained in isolated microenvironments, it is unlikely that barbering is socially transmitted between cages. Testing for hotspots controls for the fact that adjacent cages are not truly independent (i.e., they belong to other shared conditions that may not have been accounted for in our study). Thus, the hotspot outcome may be detecting effects of variables we did not or could not assess, such as belonging to the same colony, research protocol, investigator, exposure to noise or vibration, diet[10], age[20], relatedness[55], being involved in more invasive procedures, or being bred and sold by different commercial vendors[2].

Differences in the prevalence of barbering at different times of the year has not been shown before. Seasonal variation exists for prevalence of ulcerative dermatitis and aggression between males, with highest levels in mid-late summer[6,7]. Barbering levels increased over the year, peaking in autumn. However, prevalence did not return to April 2017 levels by March 2018, indicating that prevalence at different sampling dates may not be related to seasonality per se. Differences between dates could have also been related to different events occurring at each time of sampling (e.g., new personnel working with the animals, or different experiments being run). For example, we also observed a drop in prevalence in January, which could reflect reduced research activity (and therefore less stress related to disturbance from humans) following the winter holiday closure.

Number of mice in cage was highly significant as a predictor for barbering, but this does not necessarily indicate an increased likelihood of barbering due to group housing. Singly-housed mice are also known to self-barber[20]. Given that our data were collected at the cage level, cages housing more mice have an inherently higher chance of at least one barbering mouse being present. There is some evidence for an effect of higher density housing in previous studies, with overcrowded cages containing eight mice[56] or up to nine mice[39] demonstrating increased barbering. However, the number of mice per cage in this study did not reach these levels.

We also found a significantly higher prevalence of barbering in cages containing tunnels, but this is likely explained by the institutional practice of adding tunnels to cages that were exhibiting welfare problems, rather than providing them uniformly and/or preventatively. Thus, our sample of mice housed with tunnels likely consists of mice who were already provided with tunnels because they had been identified as exhibiting barbering or other welfare concerns by care staff. Experimental work shows that shelters reduce abnormal behavior in mice[24]. When mice are housed in larger cages with nesting material, a shelter, and additional rotating items (a tunnel, a gnawing bone, and plastic container), onset and overall prevalence of barbering is delayed when compared to mice housed in standard shoebox cages containing only nesting material and shelter[14]. Other recent epidemiological work did not find any effect of enrichment provision on barbering, although their enrichment conditions were larger and more well-resourced, and a low number of cages were provided with enrichment[9].

Surprisingly, rack orientation or height on the rack were not significant in the present study even though cage position on the rack was a predictor of barbering in previous work[1], and it is a known stressor potentially due to greater exposure to light, vibration, and noise[25]. Cage row and orientation of rack in the room can indeed impact mouse behavior; these factors are both significant predictors of aggression in male mice in the same dataset[57], with higher cages having more instances of fighting[7]. Type of nesting material was also not significant in predicting barbering, but all cages in our study were provided with paper and/or cotton nesting material. Presence or absence of this resource in general is likely to be important; we would expect higher prevalence of barbering in cages lacking sufficient nesting material[31].

Our identified prevalence of barbering in approximately 7.5% of cages aligns with estimates from original work[1] in this topic and with other recent studies. Young[9] found barbering in 5% of cages, also reporting a higher prevalence in females. In a descriptive survey of German animal facilities, respondents perceived barbering prevalence as less than 10% of mice in most cases, with females and C57BL/6 mice perceived as being at higher risk[2]. Respondents in this survey also reported low levels of belief that barbering could impact research findings, despite extensive literature demonstrating the contrary[58], which highlights how the scientific impacts of this behavior are likely to be overlooked.

Conclusions

Our study replicated findings from previous work showing that barbering is more likely in female mice and in breeding cages. We also identified a higher prevalence of barbering in mice housed in IVCs with corncob bedding, and in strains with a C57BL/6 mixed background. We additionally identified differences in prevalence at different points in the year and a hotspot effect in which cages adjacent to barbering mice were more likely to be barbers themselves, both of which have not been shown before. The highly significant hotspot effect indicates that there are contributing factors shared between groups of cages that go beyond what we measured in this study, serving as a reminder that other macroenvironmental variables that go unnoticed by humans could have significant impacts on research animal behavior and welfare. Our findings can be used to inform further experimental work on barbering behavior for both animal welfare and translational research.

Data availability

Data and analysis code are provided as SAS code in Supplementary Information (S1).

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Author contributions
Conceived and designed the experiments: J.H.T., J.P.G., K.P.C., B.N.G., J.A.D., S.A.F.; Performed the experiments: J.H.T.; Analyzed the data: J.A.D., J.P.G.; Figure preparation: J.P.G., A.S.R.; Contributed resources: S.A.F., J.P.G.; Writing – original draft: A.S.R, J.P.G.; Writing – revisions: all authors.

Declarations

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to A.S.R. or J.P.G.

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