MOUSE PREFERENCE FOR CAGE CHANGE INTERVAL: A SIMPLE PREFERENCE TEST TO EVALUATE HOUSING CONDITIONS FOR LABORATORY MICE

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Introduction:

For rodents, the criteria used to determine appropriate cage change intervals (i.e., days between replacing bedding with new material) are often based on institutional practices created without scientific rigor or consideration for animal welfare or health. The *Guide for the Care and Use of Laboratory Animals* had initially recommended changing small rodent cages 1 to 3 times per week (NRC, 1985). However, in the 1996 *Guide*, a revised recommendation states that the frequency of bedding change should be based on professional judgment and incorporate factors such as the number and size of the animals in the cage, the size of the cage, urinary and fecal output, and the appearance and wetness of the bedding (NRC, 1996). Other regulatory groups, such as the Canadian Council on Animal Care recommend a one-week cage change interval for rodents (CCAC, 1993). With the growing use of individually ventilated cage (IVC) systems for mice, the interval for cage changing has increased under the assumption that as long as the air quality is adequate, the degree of soiling is less relevant. Contrary to the standard one-week cleaning interval for other caging systems, the IVC system manufacturers recommend up to a 14-21 day cage change interval. This increase in interval decreases the cost of animal husbandry by decreasing the amount of bedding used and by decreasing the number of hours needed to change cages. However, following this recommendation can result in a significant increase in the degree of soiled bedding the rodent is exposed to, including fecal accumulation and moist bedding from urine. Studies evaluating mouse health as a function of extended cage changing intervals in IVCs have shown few or no pathological lesions as a function of exposure to the dirty bedding and poor air quality. However, intervals as long as 21 days can not be achieved without creating excessively moist cages with substantial fecal accumulation, extremely soiled bedding and mice whose fur appears unkempt (Reeb et al. 1998). These conditions and behavior indicate that an environment such as this may be impacting the welfare of the animal as mice are known to be fastidious about their coats and prefer to eliminate in designated areas away from their sleeping areas (Blom, 1993; Sherwin, 1996).

Choice experiments are a valuable tool in determining preferences or avoidances of housed animals. Specifically, this technique has been used to identify laboratory animal preferences and avoidances for specific housing conditions including cage size, bedding type, nesting type, group size, etc. (Blom et al., 1992). Although studies have shown that increases in cage changing interval leading to increases in ammonia levels within the cage do not affect rodent health (Reeb et al., 2000), only one study (Blom, 1993) has explored cage changing from a mouse preference perspective. In order to develop best management practices for housing laboratory mice, we evaluated mouse preference as an indicator of mouse welfare for cage change interval. We hypothesize that mice prefer less-soiled environments and when given a choice, will select these environments over more heavily-soiled environments.

Specifically, we tested the following three null hypotheses in regard to when mice can choose between various levels of soiled cage:

1. That mice have no cage preference with regard to where they build their nest;
2. That mice have no cage preference for where they spend time;
3. That age and gender do not influence cage preference.

Materials and Methods:

<u>Preference test arena</u>

Four housing conditions (i.e., unsoiled cage (0-day), 1-day soiled cage, 7-day soiled cage and 14-day soiled cage) were evaluated with the use of a multiple choice housing system, as previously described by Blom et al. (Figure 1). It consisted of an acrylic plastic center chamber measuring 5 inches square connecting four acrylic plastic topped polysulfone test cages (Alternative design, dimensions: 7.25"W x 11.5"D x 5"H). The test cages and the center chamber were connected by acrylic plastic tubes measuring 3 inches long which were 1 inch in diameter and elevated 3 inches from the bottom of the cage. Each cage contained 80 g of aspen chip bedding and either a paper towel or a Nestlet® (AnCare, Canada) as nesting material. Food in the form of Purina Mouse Chow (Purina LabDiets, 5015, Purina, U.S.A.) was provided ad libitum in small ceramic dishes on the cage bottom and tapwater was provided in Flat-bac (4 oz., Superpet, U.S.A.) water bottles inserted through a small hole in the acrylic plastic cage top. The standard wire bar lid containing food and water could was not used during taping as it obscured viewing the mice.

Prior to a preference test, individual groups were used to prepare each test condition. During this ‘soiling phase’, each cage was covered with a standard wire-bar lid to provide Purina Mouse Chow and tap water ad libitum. The following procedures were used to simultaneously create the soiled conditions. Each group was introduced to the 7-day cage for one day and then moved to the 14-day cage for 2 days. This continued until the cages had accumulated 7 or 14 days of soiling. On the day before the group was moved into the arena, mice were moved to a novel cage to create the 1-day soiled cage.

Figure 1. Testing arena for assessing mouse preference for different soiling conditions.

Execution of preference tests

The experiment started with the introduction of the mice into the center chamber and videotaping commenced. Remaining in the center chamber was discouraged by the lack of bedding, food and water. No other discouragement was provided. Two arenas were videotaped simultaneously with a cardboard visual barrier between them. The arenas were rotated 90 degrees every 24 hours for 96 hours to reduce room effects. Cages (i.e., treatments) were randomized among the 4 arena positions. Both arenas were kept behind a cloth screen to prevent visual disruption from persons entering the room and to standardize light levels to 10 Lux at cage level. The room was maintained at 18-21 C with a 12:12 hour light cycle. Experiments occurred between October 2005 and May 2006.

Mice were closely watched during the initial 5 minutes after entering the arena as some groups failed to explore the arena sufficiently to make a choice. If mice were not seen moving back through the center chamber within 5 minutes, a ramp (i.e., clear plastic insert) was added which allowed mice to move through the center chamber without stepping down. Even with the added ramp, some groups failed to explore. All groups that failed to fully explore the test arena were excluded from the analyses.

Animals

The use of animals was approved by the UBC Animal Care Committee and followed the guidelines of the Canadian Council on Animal Care. All mice were “surplus” animals obtained from several UBC modified barrier facilities which were Specific Pathogen Free for viral, bacterial, and parasitic diseases. No mice were purchased or bred for this research. Mice groups were male or female, littermates, mostly in groups of four. Most mice were transgenic knockouts on a C57bl/6 or FVB background, however some mice were non-transgenic. Ages ranged from 59 days old to 334 days old. Prior to this study, mice were housed in standard shoebox cages on either aspen chip or corncob bedding that was changed weekly.

Video-analysis

During the choice experiments, mice were videotaped 24 hr a day for 4 days using a Panasonic AG-RT850P time lapse video cassette recorder (Panasonic Corporation of North America, , Secaucus, NJ, USA). Infrared light was used during the dark period to allow for continuous recording of mouse movement. Videotapes were analyzed for mouse location and nest position with data points collected every hour on the hour from commencement of animals entering the arena for the first 48 hours. Videotapes from hour 49 through 96 were analyzed intermittently to check for a change in nest position.

Experimental design and data analysis

Data were collected for mouse groups as well as individuals. However, individual mouse observations were based on unidentified individuals. Descriptive statistics, chi-square, and Probit analyses were performed with Stata 9.1 (StataCorp LP, College Station, TX, USA). Age and gender effects were evaluated by using a multinomial probit controlling for group effects (i.e., that a mouse's choice was not independent of their group).

Results

Excluded groups

Five groups were excluded from the study due to insufficient exploration of the test arena. In general, following introduction to the arena, either all four mice moved into a cage and never left this cage or a single mouse moved into one of the four cages and remained there alone for the duration of taping (i.e., 96 hours). This behavior may have been due to avoidance for heights, as to move through the center chamber, a mouse had to step down 3 inches from the connecting tube onto the center chamber's floor. Often, mice would repeatedly explore the connecting tube but never move into the center chamber allowing them access to the other cages and therefore, to make an 'informed' choice. To help reduce the number of these groups, a clear plastic insert ("ramp") was added to the center chamber that allowed the mice to cross the center chamber without the step down. This was only added if mice did not start to explore the cages within the first 5 minutes. Following placement of the ramp, only two groups failed to explore the arena. The mice that did not explore were more likely to be older (>150 d) and male.

Dwelling times per cage

The degree of cage soiling was found to significantly affect mouse preference with mice spending significantly greater time in the 0-day cage. In our arena with its 4 locations, we would expect the dwelling times to be approximately 25% for each cage if no treatment effect was present. However, average dwelling times were significantly different (χ^2=797.89, p<0.05) than this expectation (e.g., 44% for the 0-day cage) (Table 1). While all groups showed a preference for the 0-day cage, mice under 150 days of age showed a slightly higher preference (e.g., dwelling time of 48%), whereas older animals (>150 days) showed a slightly lower preference (e.g., dwelling time of 39%). There was no difference between male and female groups in dwelling times in the 0-day cage. The probability of a mouse spending any given hour in any one cage was: 0-day= 44%, 14-day cage = 21%, 7-day cage = 19% and 1-day cage = 13%.

Table 1. Distribution of time spent (dwelling time as percentage of total time) among the four cages (first 48 hours).

Group	Unsoiled cage-0 day	1 day cage	7 day cage	14 day cage	Center chamber	Total
Age - < 150 d	48	12	18	19	3	100
Age -> 150 d	39	15	19	24	3	100
Males	44	12	27	15	2	100
Females	44	14	15	24	3	100
All groups	44	13	19	21	3	100

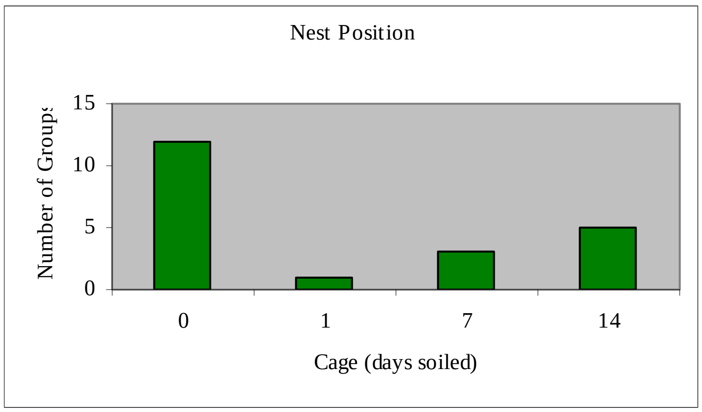
Mice tended to spend the majority of their time in the same cage that they selected to build their nest (χ^2=807.39, p<0.05). This strong effect was contributed to by the time mice spent resting in the nest and may not be an independent criterion of preference.

Nest position

Mice expressed a preference for cage soiling based on nesting behavior. Mice typically built a single new nest within 24 hours using both novel materials (i.e., from 0-day cage) as well as previously used materials from the other cages. Fifty-seven percent built their new nests in the unsoiled cage (i.e., 0-day), 24% in the 14-day cage, 14% in the 7-day cage and 5% in the 1-day soiled cage (Figure 2). Only 1 group (5%) moved their nest location during the course of the 4-day experiment. All groups built single nests and nested together.

From the above data, we calculate a χ^2 statistic of 13.10 (df_3) that is greater than the critical value of 11.34 at the 99% confidence level. Therefore, we reject the null hypothesis that mice show no preference for nest location regardless of degree of soiling.

Figure 2. Nest position by cage for all 21 groups at 96 hours.



To show preference for the 0-day cage, we compared this cage to all other cages grouped together. The chi-square value in this case equaled 11.57 (df_1). The critical value at the 99% confidence level is 6.63, so we can clearly reject the null hypothesis that mice have no cage preference with regard to where they build their nest.

Age and gender effect

There were no differences in the characteristics of mice that preferred the 0-day cage to the 1- or 7-day cages. However, mice that preferred the 14-day cage tended to be older females.

Effects of the ramp addition

There is also some evidence that groups given a ramp (i.e., mice that initially did not explore the arena) were more likely to spend time in the 0-, 1- and 7-day cages, while not having a ramp increased the probability of spending time in the 14-day cage by almost 22%.

Discussion:

Guidelines governing rodent cage change intervals have traditionally been based on criteria other than animal welfare or health. In order to gain insight into the animal's preference, we evaluated whether the level of cage soiling affected mouse choice for where they build their nest and spend their time. This study demonstrates that mice do express a preference for a clean cage versus a soiled cage. When given a choice, the majority of groups nested and spent the largest proportion of their time in the unsoiled cage (e.g., a novel environment). In addition, we found it interesting that the second most preferred cage was the 14-day cage and that the most recently occupied cage (1-day cage) was the least preferred for nesting and dwelling. This is contrary to the study by Blom (1993) which found that increased degrees of soiling were closely associated with increased cage avoidance and that mice prefer cages that were familiar. Based on Blom's study, we expected mice to prefer the 0-day, 1-day and 7-day cages over the 14-day cage. It should be noted that all cages, including the 14-day cage, were not excessively moist and had only a moderate amount of soiling. This may have been due to group size as well as the amount of bedding in each cage. Although not evaluated in this study, it is possible that the mice continued to use the 1-day cage as a latrine, based on the most recent soiling/scent marking in this cage. This would account for the 1-day cage being the least preferred for nesting or dwelling.

Based on previous studies, it is normally assumed that stress expressed by rodents during and soon after a cage change is because of the novel environment the animal finds itself in (e.g., clean cage). However, we found mice expressed a strong preference for a novel environment (i.e., 0-day cage). Studies on rodent stress have demonstrated that cage changing causes elevations in heart rate, mean arterial blood pressure and stress behaviors (Duke et al, 2001; Sharp et al., 2003). In addition, daily cage changing has been shown to decrease mass gain and is often used as a stressor in rodent studies. However, it is very difficult to separate the effects of introducing a mouse to a novel cage and the actual physical handling of the mouse. In this study, mice had the option to explore a novel environment (i.e., the unsoiled cage) at will and leave if they chose, without the complication of additional handling. Therefore, we conclude the stress attributed to exposure to a novel environment is indeed more closely related to the physical disturbance of the animals and not the novel environment per se.

It appears that groups made ‘common’ decisions regarding where they spent their time and where they built their nest. However, it was clear that specific individuals within a group were more prone to exploratory behavior than others and may have had more influence in the group decision making process. Following exploratory behavior, some groups began nesting in one cage but then for unknown reasons changed locations. All mice nesting together in one cage was expected as mice generally show a preference to be in close proximity to other conspecifics, even males that have been housed singly (Vandenbroek et al., 1993; van Loo et al., 2001).

Mice show a strong preference for nesting material and have been observed choosing nesting material over contact with another mouse (Blom, 1993; Sherwin, 1997; Olsson and Dahlborn, 2002). In our test arena, only the 0-day cage contained new nesting material; all others were left with the old nest intact. Therefore, the preference for the 0-day cage may be based on their preference for fresh nesting material and not the cleanliness of cage. However, nesting material could be moved throughout the arena, and for those mice that chose to nest in the 0-day cage, they actually brought used nesting material from all other cages. In addition, mice that chose to nest in a cage other than the 0-day cage moved nesting from the 0-day cage to their ‘nest’ cage. Therefore, degree of soiling appears to be of more influence than nesting material in cage selection.

There was little effect of age and sex in this study. Although not significant, older female mice had a slight preference for the 14-day cage. This may be due to the females creating less soiled cages than males or younger mice by urinating or defecating in specific locations, thereby keeping the remainder of the cage cleaner. This finding was contrary to that of Blom (1993) who concluded that older animals were more likely to avoid more soiled cages.

This study indicates a preference by mice for a clean, unsoiled cage based on dwelling times and location of nest. Therefore, from an animal welfare perspective, housing mice in cages with longer change intervals should be further evaluated, especially considering the increased use of IVC systems. Future tests should evaluate the strength of preference by mice for clean cages and whether the simple addition of clean nesting material into a soiled cage can affect preference. While it is imperative to standardize all aspects of housing for research rodents, it is equally important to ensure that those housing standards provide the most optimal welfare for the animal.

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