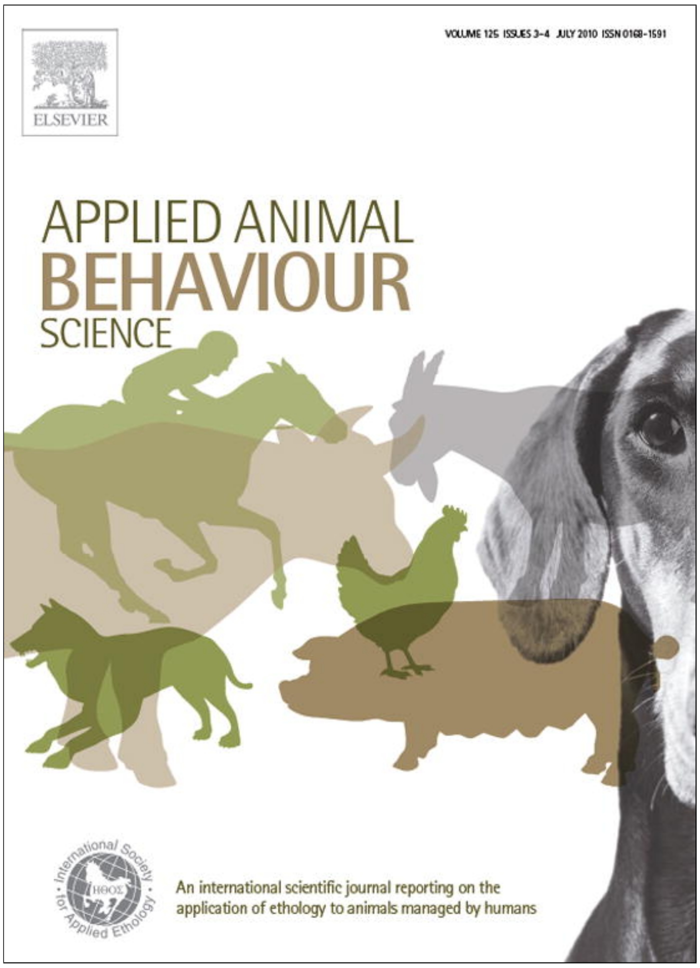
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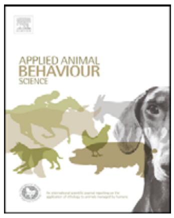

Preference of Syrian hamsters to nest in old versus new bedding

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ABSTRACT

This study tested whether Syrian hamsters (*Mesocricetus auratus*) have an aversion to old bedding (up to 14 days) by offering them the option to nest in a new cage. A secondary goal was to assess the relative value of shelters by testing whether the tendency to nest in the new cage was reduced when a shelter was present in the old cage. Individual hamsters were placed in two cages connected by a tunnel, and left to familiarize themselves with this set-up for 10 days. Then the bedding was changed in each cage, and for the next 3, 9, or 14 days the tunnel was blocked and each hamster lived in only one of the cages, either with or without a shelter (PVC pipe) present in that cage. Then the tunnel was unblocked and for the next 3 days the position of each hamster's nest in either of the two cages was noted. After 3, 9, and 14 days in the old cage, respectively 10, 11, and 8 out of 15 males and 6, 9, and 3 out of 15 females never nested in the new cage, whether the old cage had a shelter or not. Only 2, 1, and 4 out of 15 males, and only 5, 3, and 5 out of 15 females nested in the new cage more than in the old one in the absence of shelters. Of those males that nested in the new cage at least once, three out of five, three out of four, and five out of seven nested in the new cage less often when a shelter was present in the old cage than when no shelter was present. For females, the corresponding numbers were 8 out of 9, 5 out of 6, and 11 out of 12. These results indicate that access to a new (though still familiar) cage with fresh bedding holds only a small attraction for nesting hamsters, at least when their current bedding is up to 14 days old, and that shelters are valued.

1. Introduction

Syrian hamsters, *Mesocricetus auratus*, are common study animals in biomedical and behavioural research. In Canada for example (Canadian Council on Animal Care, 2010), 5724 hamsters were used for research purposes in 2008, making them the fourth most used rodents after mice (1,053,946), rats (305,819) and guinea pigs (28,810). So far, hamster welfare has been studied in terms of their social housing (Arnold and Estep, 1990), nest boxes (Ottoni and Ades, 1991), cage floor preference (Arnold and Estep, 1994; Arnold and Gillapsy, 1994), cage dimensions (Kuhnen, 1999; Fischer et al., 2007), environmental enrichment (Reebs and Maillet, 2003), running wheels (Mrosovsky et al., 1998; Gebhardt-Henrich et al., 2005; Reebs and St-Onge, 2005), and bedding material (Hauzenberger et al., 2006; Lanteigne and Reebs, 2006). Many aspects of hamster welfare, however, remain open to study. Among them are the values hamsters assign to familiar bedding and to shelters.

The first goal of the present study was to measure the preference of Syrian hamsters for clean versus lived-in bedding during their resting phase. Hamsters are nocturnal animals that, in captivity, spend most of their nighttime running in wheels (Reebs and Maillet, 2003) and almost all of their daytime sleeping in a nest usually made of bedding material (Kuhnen, 2002; Lanteigne and Reebs, 2006).

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Replacing soiled bedding with new material is a standard animal husbandry procedure, but the frequency at which this should be done can be a matter of debate. Health-related parameters such as the build-up of ammonia levels (Gamble and Clough, 1976; Schoeb et al., 1982; Smith et al., 2004) should figure prominently in the decision, and generally such considerations tend to promote relatively high frequencies of bedding change. However, animals may find cage cleanings stressful (for hamsters, see Gattermann and Weinandy, 1996–1997; and for other rodents, see Gärtner et al., 1980; Saibaba et al., 1996; Duke et al., 2001; Balcombe et al., 2004) and they may also value the familiarity of their scented environment, including their nest (for an example of the importance of scents for hamsters, see Johnston et al., 1993). Such considerations would argue against a high frequency of bedding change. In this regard, preference tests can provide useful information about the value the animals themselves attribute to old versus new bedding (Blom et al., 1993). In one rare example of a preference test applied to old and new bedding, Burn and Mason (2008) found that rats did not show any preference between a cage with bedding changed every 3–4 days or one with bedding unchanged for 18 days, with the conclusion that frequent cage cleanings did not cause olfactory disruption in rats. Here, in a variant of this approach, we offered hamsters a choice between nesting in a new clean cage (with which they were already familiar) or remaining in the cage they had lived in for the past 3, 9, or 14 days. These durations span a range from roughly half to double the weekly bedding changes recommended for hamsters by the Canadian Council on Animal Care (1984).

The second goal of the study was to provide data on the value of shelters in Syrian hamsters. In our laboratory we recently found that hamsters prefer to use cages with a wheel and a shelter (a PVC pipe closed at one end) over cages with only a wheel, and that they nest directly in such a shelter about 50% of the time (Veillette and Reebs, submitted). This is evidence that captive hamsters value shelters, but additional evidence could be provided by pitting shelters against other resources, such as access to new bedding, in preference tests. We therefore conducted our bedding choice experiments both with and without the presence of a shelter in the cage with soiled bedding. We predicted that if shelters are valued by hamsters, then use of the cage with new bedding would be reduced in the presence of a shelter in the cage with soiled bedding.

2. Materials and methods

2.1. Animals and materials

All experiments were approved by the Université de Moncton's animal care committee (protocol # 07-10). Syrian hamsters (15 males and, in a later replicate, 15 females, all 60 days old) were purchased from Charles River Canada (Saint-Constant, Québec, Canada). They had no prior experience with shelters. Upon arrival in the laboratory, they were individually placed in sets of two polypropylene cages (Nalgene®, Nepean, Ontario, Canada; 42 cm long × 22 cm wide × 21 cm high; opaque and white) connected by a tunnel (Habitrail®, Montreal, Quebec, Canada; 18 cm long × 6.4 cm diameter; transparent). Each cage had a running wheel (Nalgene®, F-size for rats; 35 cm diameter), bedding material (Canada Pine Shavings®, Saint-Nicolas, Quebec, Canada; 1000 mL), and access to food pellets (Pro Lab®, Lab diet, Moncton, New Brunswick, Canada) and distilled water (bottled) in an overhead hopper (Fig. 1). Room temperature was set at 20 °C and humidity was between 40 and 55%. Lights came on at 08:30 h and went off at 22:30 h, for a light:dark cycle of 14:10 h. Light intensity at cage level was about 45 lx (measured with a Lunasix 3 photometer, Gossen®, Nuremberg, Germany) and was uniform for all cages. Such conditions (except for the double cage) are standard for hamsters kept in captivity (Canadian Council on Animal Care, 1984).

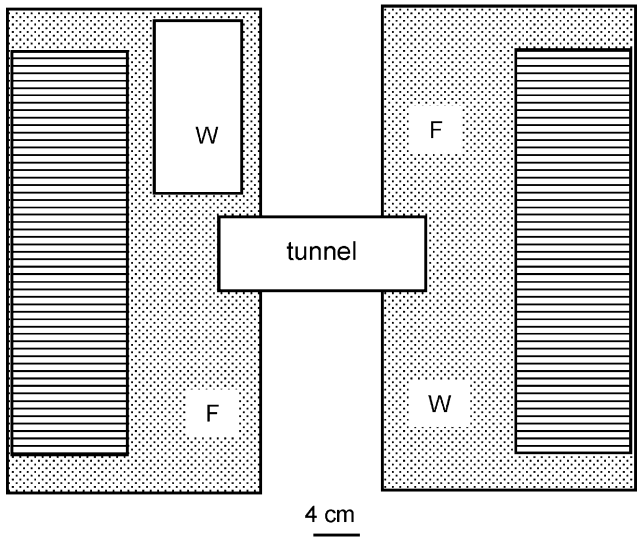

Fig. 1. Overhead view of the two-cage set-up given to each hamster. The lined area represents the wheel placement; the grey area represents the cage bedding. W and F are for water and food, respectively. The white square shows the placement of the shelter when present in the cage.

The hamsters were left to familiarize themselves with their two-cage set-ups for 10 days. (They explored both cages within a few minutes of their being introduced to the set-up, and readily used both cages for nesting thereafter.) For half of this 10-day period a shelter was present in both cages, and for the other half neither cage had a shelter. The shelters were an acrylonitrile butadiene styrene (ABS) pipe (obtained in the plumbing section of a local hardware store), black and opaque, 15 cm long and 7.6 cm in diameter, and closed at one end with an ABS cap. This was the shelter shown by the previous study in our laboratory (Veillette and Reebs, submitted) to be preferred by hamsters over the absence of a shelter and over a variety of other shelter types.

2.2. Methods

At the end of the 10-day familiarization period, cages were cleaned, bedding was changed (1000 mL, compacted), and each hamster was placed in one cage (either right or left, determined at random). Access to the second cage was blocked for either 3, 9 or 14 days, during which bedding was not changed. Then the tunnel was unblocked and the animals were left to use either cage for the next 3 days. (To avoid disturbing other experimental groups in the room, access to both cages after the 14-day treatment had to be lengthened to 5 days, but only the first 3 days were used in the analyses.) During these 3 days, called the observation period, cages were unobtrusively inspected twice a day, during the daytime, to determine which cage each hamster used for nesting. (Hereafter, "old" refers to the cage where the bedding was soiled, and "new" to the cage where the bedding was clean.) Nesting was defined as the animal resting or sleeping with a mound of bedding around it. In 4% of all observations, hamsters were moving around, eating, or drinking instead of nesting, and these observations were discarded. Cage use was noted twice a day rather than only once a day to allow for the small possibility of multiple nest use. In the end, nest changes from one cage to the other during the same day occurred only 5% of the time.

At the end of the observation period, animals were weighed, cages were cleaned, new bedding was placed in the cages, and a new treatment was begun. Care was taken so that cage changes between treatments did not occur during the observation periods of other hamsters in the room, so as to minimize disturbance. Before being discarded, bedding from the new cage was compacted and placed in a 1000 mL beaker to measure its volume to the nearest 50 mL. This was done to evaluate the possible transfer of clean bedding from the new cage to the old one. (Instead of moving to the new cage, an animal could transfer clean bedding to the old cage, something hamsters can easily do using their ample cheek pouches.) This approach, however, only provided a measure of net transfer; absolute transfer in both directions could not be ascertained.

Eventually all animals were tested after 3, 9, or 14 days in their old cage, in both a control and an experimental situation. In the control situation, use of the new cage was measured when no shelter was present in the old cage, neither before nor after the new cage became available. In the experimental situation, use of the new cage was measured when a shelter was present in the old cage both before and after the new cage became available. Order of presentation of the six situations was: control 3–9–14 and experimental 3–9–14 for four animals of each sex; control 14–9–3 and experimental 14–9–3 for three animals of each sex; experimental 3–9–14 and control 3–9–14 for four animals of each sex; experimental 14–9–3 and control 14–9–3 for four animals of each sex. This balanced variation between animals was used to minimize the risk of systematic treatment order effects (these effects were also tested statistically).

2.3. Statistical analysis

The proportion of observations when the nest was in the new cage was the response variable. Bedding age in the old cage (3, 9, or 14 days) and shelter presence (control or experimental) were the explanatory variables. Order of bedding age presentation (either 3–9–14 or 14–9–3) and order of shelter presentation (either control first then experimental or experimental first then control) were considered as possible confounding variables.

First, a Wilcoxon signed rank test was used to determine if there was an effect of shelter presence on use of the new cage (the no-shelter control was paired with the shelter treatment for each individual and bedding age). Next, the effects of bedding age, order of bedding age presentation, and order of shelter presentation were introduced in a model. The first method used was the Mantel–Haenszel statistic for general association. If this was significant, generalized estimating equations (GEE, with the GENMOD procedure) with repeated measures (individuals as the repeated measures) when possible (i.e., if the Hessian matrix was positive definite) were used with a binomial distribution and a logit link to test for effects on the response variable. A GEE procedure was also used to test for sex differences in the number of individuals that used the new cage at least once, or that used the new cage more than the old one.

For differences in bedding volume in the new cage between the start and end of an observation period, only the cases when an individual hamster did not nest in the new cage were considered. If there was less bedding in the new cage at the end than at the beginning of an observation period, a positive value was given (considered a net transfer to the old cage). If there was a higher volume than at the start, a negative value was given (considered a net transfer from the old cage) but this happened only 4 times out of 131 measures. Data were rank transformed since no transformations satisfied the conditions of normality and homogeneity of variance of the residuals (as tested with the Komolgorov–Smirnov test). A nonparametric ANOVA (general linear model procedure) was used to test for the effects of bedding age, shelter presence, order of bedding age presentation, and order of shelter presentation.

All alpha levels were set at 0.05. *P* values between 0.05 and 0.10, though non-significant, were nevertheless considered a trend and are reported as such. All analyses were performed with SAS, version 9.1 (SAS® software, 2007). Means are given along with standard errors.

3. Results

3.1. Males

Most male hamsters (10, 11, and 8 out of 15 in the 3-, 9-, and 14-day treatments, respectively) never nested in the new cage, irrespective of the presence or absence of a shelter in the old cage. Of those that nested in the new cage at least once, roughly half still nested in the old cage more often than in the new one (data points below 0.5 in Fig. 2). Only two (3 days), one (9 days) and four (14 days) males out of 15 nested in the new cage more than in the old one in the control situation (i.e., no shelter present). These proportions correspond to $P = 0.004$, 0.0005 and 0.059 respectively on a Wilcoxon signed rank test.

Of those males that nested in the new cage at least once, three out of five (3 days), three out of four (9 days), and five out of seven (14 days) nested less often in the new cage when a shelter was present in the old cage as compared to when no shelter was present (Fig. 2). The latter two proportions correspond to $P = 0.11$ on a Wilcoxon signed rank test.

Table 1 shows the average use of the new cage for nesting according to the various experimental conditions, including those animals that never nested in the new cage.

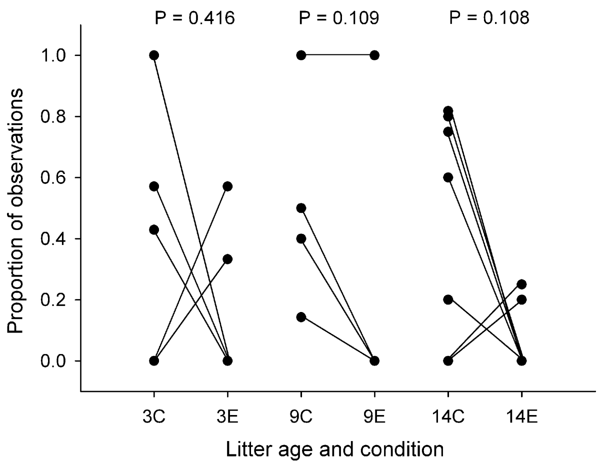

Fig. 2. New cage use for nesting by male hamsters when a new cage was made available after 3, 9, or 14 days spent in their old cage, with a shelter either absent (control = C) or present (experimental = E) in the old cage. Each hamster acted as its own control, as shown by the lines joining dots. Only the results from the animals that used the new cage at least once are given. *P*-values show the results of a Wilcoxon signed rank test.

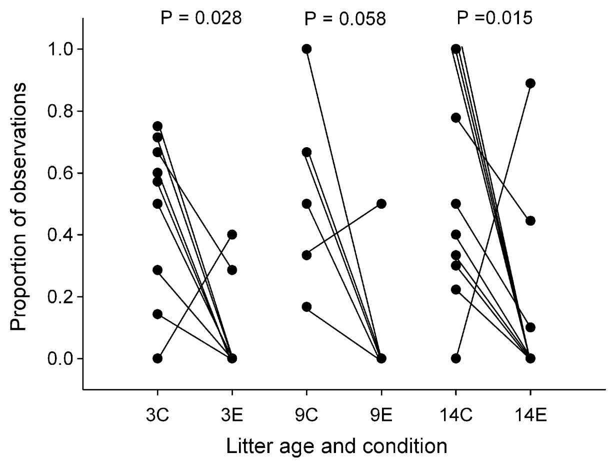

Fig. 3. New cage use for nesting by female hamsters when a new cage was made available after 3, 9, or 14 days spent in their old cage, with a shelter either absent (control = C) or present (experimental = E) in the old cage. Each hamster acted as its own control, as shown by the lines joining dots. Only the results from the animals that used the new cage at least once are given. *P*-values show the results of a Wilcoxon signed rank test.

The results of the Mantel–Haenszel test were df = 7, value of 46.5 and $P < 0.0001$. This meant that either the different orders of presentation, bedding age, or shelter presence had an influence on the use of the new cage for nesting. The GEE model revealed that in fact neither the order of bedding age presentation ($F_{(1)} = 0.003$, $P = 0.996$) nor the order of shelter presentation ($F_{(1)} = 0.03$, $P = 0.87$) had a significant effect on new cage use. There was also no significant effect of bedding age ($F_{(2)} = 0.08$, $P = 0.96$) and no interaction effects ($F_{(2)} = 3.32$, $P = 0.191$). However, a trend for an effect of shelter presence ($F_{(1)} = 3.16$, $P = 0.076$) was found: use of the new cage tended to be less when a shelter was present in the old cage.

The ANOVA testing the effects of bedding age, shelter presence, order of bedding age presentation, and order of shelter presentation on bedding transfer yielded a significant overall model ($F_{(23,49)} = 2.22$, $P = 0.0096$, $R^2 = 0.51$). Bedding age ($F_{(2)} = 4.11$, $P = 0.02$) was the only significant main effect; there was significantly more transfer of bedding to the old cage after 9 and 14 days (57 ± 10 and 41 ± 14 mL, respectively) than after 3 days (20 ± 4 mL). There was also a trend ($F_{(1)} = 3.22$, $P = 0.08$) for more transfer when a shelter was in the old cage (45.6 ± 5.4 mL) as compared to when no shelter was present (32.6 ± 12.8 mL).

3.2. Females

Some females never nested in the new cage (6, 9, and 3 out of 15 in the 3-, 9-, and 14-day conditions, respectively), irrespective of the presence or absence of a shelter in the old cage. Overall these proportions are almost significantly lower than for males ($\chi^2 = 3.64$, $P = 0.056$). As in males, roughly half of those animals that nested in the new cage at least once still nested in the old cage more than in the new one (data points below 0.5 in Fig. 3). Only 5 (3 days), 3 (9 days) and 5 (14 days) females out of 15 nested in the new cage more than in the old one in the control situation (no shelter present). Overall these proportions are not significantly different than for males. They correspond to $P = 0.15$, 0.02, and 0.15 respectively on a Wilcoxon signed rank test.

Of those females that nested in the new cage at least once, 8 out of 9 (3 days), 5 out of 6 (9 days), and 11 out of 12 (14 days) nested less often in the new cage when a shelter was present in the old cage as compared to when no shelter was present (Fig. 3). On a Wilcoxon signed rank test, these proportions correspond to $P = 0.03$, 0.06, and 0.02, respectively.

Table 2 shows the average use of the new cage for nesting according to the various experimental conditions,

Table 1
Percentage of new cage use for nesting by 15 male hamsters when no shelter (control = C) or a shelter (experimental = E) was present in the old cage. Each hamster acted as its own control and was tested after 3, 9, and 14 days in its old cage.

Condition	Order of bedding age presentation	*N*	% use of new cage	
			No shelter in old cage (C)	Shelter in old cage (E)
E first	14–9–3	4	22.9	12.5
	3–9–14	4	27.3	6.1
C first	14–9–3	3	13.8	0
	3–9–14	4	15.2	0

Table 2
Percentage of new cage use for nesting by 15 female hamsters when no shelter (control = C) or a shelter (experimental = E) was present in the old cage. Each hamster acted as its own control and was tested after 3, 9, and 14 days in its old cage.

Condition	Order of bedding age presentation	*N*	% use of new cage	
			No shelter in old cage (C)	Shelter in old cage (E)
E first	14–9–3	4	40.0	5.3
	3–9–14	4	42.3	0
C first	14–9–3	3	28.8	14.3
	3–9–14	4	20.6	6.7

including the animals that never nested in the new cage. The results of the Mantel–Haenszel test were df = 1, value of 68.12 and $P < 0.0001$. Therefore, as with males, either bedding age, shelter presence, or the different orders of presentation had an influence on the use of the new cage. The GEE model showed that, in fact, neither bedding age ($F_{(2)} = 4.55$, $P = 0.10$) nor the order of presentation of bedding age ($F_{(1)} = 0.57$, $P = 0.45$) had an effect (though, at $P = 0.10$, bedding age showed a trend towards significance; there was a tendency for more nesting in the new cage when the soiled bedding was older). Shelter presence and order of presentation of shelters, on the other hand, were significant when combined into one treatment ($F_{(3)} = 7.95$, $P = 0.05$). Independent contrasts revealed that the effect was due entirely to the presence of shelter and not to shelter presentation order ($P = 0.004$ versus $P = 0.47$, respectively). A final model thus included only shelter presence and bedding age, the two variables previously shown to be significant or nearly significant. Only shelter presence ended up being significant in this model ($F_{(1)} = 5.82$, $P = 0.016$), meaning that new cage use for nesting was lower when a shelter was present in the old cage.

Females did not show any significant patterns of bedding transfer. The ANOVA testing the effects of bedding age, shelter presence, order of bedding age presentation, and order of shelter presentation yielded a non-significant overall model ($F_{(21,38)} = 1.32$, $P = 0.22$, $R^2 = 0.42$). Females transferred on average 29 ± 7 mL (3 days), 28 ± 8 mL (9 days) and 47 ± 11 mL (14 days) of bedding. Mean transfers in the shelter and no-shelter conditions were 32 ± 9 mL and 34 ± 6 mL, respectively.

4. Discussion

A majority of male hamsters (53–73%) and a number of females (20–60%) never nested in the new cage when it became available, even when the old cage had 14-day-old bedding. Moreover, about half of the animals that used the new cage for nesting did so less than 50% of the time during the 3-day observation period. When no shelter was present (and thus only bedding age could distinguish the cages), the number of animals that used the new cage more than the old one was only 1–4 males and 3–5 females out of 15. These results point to a relatively low motivation to use new bedding for nesting, and an overall preference for old bedding. It could be argued that low use of the new cage for nesting was simply an expression of neophobia rather than a preference for old bedding, but this seems unlikely given that all hamsters had been familiarized with the two-cage set-up for 10 days prior to the experiments, that all animals were exposed to six treatments sequentially (the early treatments should have contributed to familiarization), and that hamsters do not hesitate to explore and nest in multiple cages when first placed in such set-ups (personal observations in this study and in others in our laboratory). Low motivation to use new bedding means that sanitary and health considerations may be the only reasons justifying weekly cage changes in hamsters. The animals themselves may prefer longer intervals up to at least 14 days. In fact, hamsters have been shown to find cage cleaning one of the most stressful routine laboratory procedures (Gattermann and Weinandy, 1996–1997). It remains to be determined whether the negative effect of disturbance can outweigh health benefits enough to recommend lengthening the interval between cage changes, but at least the hamsters' own preference cannot be invoked when advocating weekly changes.

The conclusion that hamsters prefer to nest in old rather than new bedding is somewhat tempered by the fact that male hamsters transferred more new bedding into their old cage in the 9- and 14-day conditions than in the 3-day condition, an indication that they may value new cage bedding more when their current cage bedding is older. The amounts transferred, however, were small (between 30 and 50 mL on average). Moreover, we could not ascertain what the hamsters did with the transferred bedding, nor the exact amounts that may have been transferred in both directions between the new and old cages. Future experiments might use marked bedding material (with different colours, for example, if bedding colour can be shown not to affect hamster behaviour) to better evaluate bedding transfer in multiple cage set-ups and in choice experiments.

Our conclusion about the lack of motivation to use new bedding applies only to nesting activity. We did not observe hamster activity at night, when the animals were undoubtedly wheel running. We did notice that fecal pellets were present in both the new and old cage, indicating that the hamsters did not avoid the new cage at night and that they probably used both wheels. Reebs and Maillet (2003) also found that hamsters use multiple wheels when they are placed in multiple cage set-ups.

For thoroughness we tested both males and females, but even though male and female hamsters sometimes differ in aspects relevant to animal welfare issues (e.g., Beaulieu and Reebs, 2009), we did not *a priori* expect any particular differences between them in this case. We nevertheless found a statistical trend ($P = 0.0564$) for more females than males to nest at least once in the new cage when it became available. Larger sample sizes are needed to confirm this result. In a previous study (Veillette and Reebs, submitted), females were found to use deep cage bedding as a nesting site more often than males. It might be interesting to determine if females value well insulated nests (and thus, perhaps, more and cleaner cage bedding) more than males, possibly for reasons ultimately linked to the rearing of young.

As per our prediction, shelters in the old cage increased the attraction of that cage when it became possible for the hamsters to move to a new one. Too few males used the new cage to make our test sufficiently powerful (the GEE model only revealed a trend at $P = 0.076$), yet it is striking that 11 of the 16 lines in Fig. 2 go down to zero in the experimental condition, suggesting that shelters kept these animals in their old cage for nesting. The statistical trend ($P = 0.08$) for more bedding transfer by males to their old cage when a shelter was present there is also indicative that hamsters prefer to stay in their shelter. In the case of females, the signed rank test showed significant differences between the control and experimental conditions for all treatments, and most of the females that used the new cage at least once did so only when no shelter was present in the old cage (20 of the 26 lines in Fig. 3 go down to zero in the experimental condition). Overall, these results indicate that hamsters value the use of their shelters for nesting more than a cage with new, clean bedding. To determine whether shelters could also reverse the attraction of a lived-in cage, the present experiment should be replicated with shelters in the new cage instead of the old one.

5. Conclusions

Access to a new cage with clean bedding holds a relatively small attraction for hamsters, at least when their current cage bedding is up to 14 days old. This suggests that hamsters are not inconvenienced by old bedding, that they actually prefer it for nesting, and that weekly cage changes are not necessary (strictly in terms of the hamsters' preference; sanitary considerations are another matter). Moreover, the small attraction to a new cage with clean bedding is further reduced when a shelter is in the old cage, and this constitutes evidence that hamsters value shelters.

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