Preference for bedding material in Syrian hamsters

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Summary

This study aimed to determine whether Syrian (golden) hamsters, Mesocricetus auratus, prefer certain bedding materials and whether bedding material can affect paw condition. body weight gain and wheel-running activity. In a first experiment, 26 male hamsters had access to two connected cages, each cage containing a different bedding material (either pine shavings, aspen shavings, corn cob or wood pellets). In a second experiment, 14 male hamsters had access to four connected cages that contained the different bedding materials and also a piece of paper towel to serve as nest material. In a third experiment, 30 male hamsters were each placed in a single cage, 10 of them with pine shavings, 10 with aspen shavings and 10 with corn cob, and they were monitored for 50 days. Significant preferences in the first experiment were: pine shavings over aspen shavings, corn cob over wood pellets, pine shavings over corn cob and aspen shavings over wood pellets (aspen shavings versus corn cob was not tested). However, there was no significant preference expressed in the second experiment, suggesting that the general preference for shavings in the first experiment was based on bedding material suitability as a nesting material. No significant effect of bedding material on paw condition, body weight gain and wheel-running activity was detected. None of the four bedding materials tested in this study can be judged to be inappropriate in the short term if nesting material is added to the cage and if the litter is changed regularly.

Keywords Syrian hamster; golden hamster; bedding material; nesting material; choice; preference; animal welfare

Captivity conditions must satisfy the basic needs of laboratory animals and ensure their physical, physiological and psychological welfare. Bedding material is an important component of a laboratory animal's environment. It provides sanitary conditions by absorbing body wastes. Inasmuch as possible, it must not contain pesticides or carcinogenic substances (Weisbroth 1979, Raynor *et al.* 1983, Arnold & Estep 1994, Van de Weerd *et al.* 1996). It must minimize the production of ammonia by bacteria (Perkins & Lipman 1995), as an excess of

Correspondence: Stéphan Reebs. Email: reebss@umoncton.ca ammonia can harm the trachea of animals (Gambel & Clough 1976). Dust content of bedding material can also be harmful and cause allergies, respiratory problems and cancers (Raynor *et al.* 1983, Arnold & Estep 1994, Potgieter & Wilke 1996).

Bedding may also double as a nesting material and as a substrate on which to walk. Comfort and suitability for nest construction and body contact can therefore become criteria in the choice of appropriate bedding material. Animal preferences for bedding material over others have sometimes been interpreted in this light (Blom *et al.* 1996, Ago *et al.* 2002). Psychological needs such as comfort are often studied through preference tests.

There has been a fair amount of research on the preference for bedding material exhibited by rats (Blom et al. 1996, Van de Weerd et al. 1996, Ras et al. 2002) and mice (Iturrian & Fink 1968, Mulder 1975, Blom et al. 1996, Ago et al. 2002). In contrast, we are aware of no such research on Syrian (golden) hamsters, in spite of this animal's popularity in chronobiological as well as some types of biomedical studies. In fact, welfare studies on hamsters seem to be limited to Arnold and Estep (1994) on cage floor choice, Mrosovsky et al. (1998) and Reebs and St-Onge (2005) on running wheel choice, Kuhnen (1999) on cage size, and Reebs and Maillet (2003) on environmental enrichment. In the present study, we investigated whether hamsters have a preference for different types of bedding material. We also looked at short-term effects of bedding material on paw condition, body weight gain and wheel running.

Experiment I

Material and methods

All experiments were conducted under approval by the Université de Moncton's Animal Care Committee (protocol nos 03–01 and 04–08).

The preference apparatus consisted of two translucent white cages (Nalgene, F-size for rats, $47 \times 26 \times 20$ cm) made of polypropylene and connected by a plastic tube (Hagen's Habitrail; 6 cm diameter, vertical access tunnel 19 cm long, connecting tunnel 37 cm long). Each cage had a water bottle with distilled water. Food pellets for laboratory rodents (Hagen) were distributed on the floor of each cage and replaced as needed. In each cage was a running wheel (Nalgene, F-size for rats, 35 cm in diameter) connected to a computer system for recording daily running activity. Bedding material was spread on the floor of each cage. Thirteen such apparatus were kept side by side on shelves in a single room kept at $21 \pm 1^{\circ}$ C, under a 14:10 h light:dark cycle. Light was provided by two incandescent lights on the ceiling of the

room, resulting in an intensity of about 100 lux within the cages.

Four common commercial bedding materials were tested. Two were made of wood shavings (one aspen, the other pine, both from Rolf C Hagen Inc). Two more were made of granules (one consisting of wood pellets, i.e. small cylinders of wood 6 mm in diameter and 5-25 mm long, from Kaytee Co; the other made of bits of corn cob, about 2-5 mm in diameter, from Rolf C Hagen Inc). A fifth type of commercially available litter, rather granular in type (heat-treated hardwood chips, 'Beta-Chips', Northeaster Products) was not tested because this litter is used by the farm that raises the hamsters we purchase, and we wanted to minimize possible bias caused by previous experience.

In a first experiment, we pitted the two shaving types against each other, and did the same with the two granular types. Thirteen male hamsters, 60 days old, were purchased from Charles River Canada (the number of hamsters corresponded to the maximum number of two-cage set-ups that could be fitted in the room). Upon arrival in the laboratory, each hamster was placed in a preference apparatus, either in the right- or the left-hand side, determined at random. At that time, the cages did not contain any bedding material. About 24 h later (sufficient time for the hamsters to learn how to use the connecting tubes), 1 L of bedding material was added to each cage, irrespective of where the hamster was at that time. For seven of the hamsters, one cage (determined at random) received pine shavings and the other cage received aspen shavings; the other six hamsters received wood pellets and corn cob. Twelve days later, the litter was changed: the hamsters that had received the shavings pair now got the granular pair, and vice-versa. Twelve days after that, the whole cycle was repeated one more time (second trial). Thus each hamster was twice given a choice between aspen and pine shavings, and twice between wood pellets and corn cob.

A second experiment was conducted with 13 new male hamsters, as above except that the pairings involved pine shavings versus corn cob (the two 'winners' of the pairings in the first experiment) and aspen shavings versus wood pellets (the two 'losers'). Here, all hamsters began the experiment with the same pairing: pine shavings versus corn cob.

The position (cage) of each hamster, its nest, its food, its latrine (accumulation of urine) and its faeces was noted once a day, during the light period of the light-dark cycle. The number of running wheel revolutions was also tallied daily. The days that preceded and followed litter changes were discarded, so that only 10 days of data were used in the analysis. Depending on the experiment and trial, 2-6 hamsters slept in the tunnel on 1-4 days, and such data were not used in the analysis.

All statistical tests were conducted with SPSS for Windows. Significance level was set at P = 0.05.

Results

Few hamsters were found in the same cage on all 10 days (Figures 1 and 2). If we define a preference as being more than 50% of all positions observed, then in the test of pine versus aspen shavings (Figure 1a), eight out of 13 hamsters preferred pine over aspen in the first trial (P = 0.2905 on a binomial test)and 10 out of 13 in the second trial (P = 0.0461). In the first trial, three hamsters transferred pine shavings into the aspen shavings cage and made a nest out of it (the volume transferred was not measured, but it was sufficient to ensure that the body of the sleeping hamster was only in contact with that material). If we take this as a preference for pine, then 12 out of 13 hamsters preferred pine over aspen in the first trial (P = 0.0017). There were no transfers during the second trial, and never any transfer of aspen shavings.

In the test involving corn cob versus wood pellets (Figure 1b), eight out of 13 hamsters preferred corn cob in the first trial (P = 0.2905). This number rises to 10 out of 13 if transfers (all of which were of corn cob into the wood pellet cage) are taken into account (P = 0.0461). In the second trial, 11 out of 13 (P = 0.0112) preferred the corn cob; this becomes 12 out of 13 if transfers are taken into account (P = 0.0017).

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In the test involving pine shavings versus corn cob (Figure 2a), eight out of 13 hamsters preferred pine shavings in the first trial (P = 0.2905). This number rises to 10 out of 13 if transfers (all of which were of pine shavings into the corn cob cage) are taken

(a) 100



Figure 2 Percentage of days (out of a maximum of 10) spent by 13 hamsters on pine shavings versus corn cob on two trials separated by 12 days (a) and for aspen shavings versus wood pellets on two more trials separated by 12 days (b). Open symbols indicate animals that had transferred bedding from one cage to the other (the data show the percentage of days spent in the cage from which the bedding was transferred, which still contained a good portion of that bedding, and not the percentage of days spent nesting on the bedding irrespective of the cage where it was found). Some data points were offset slightly to distinguish each of the 13 lines

into account (P = 0.0461). In the second trial, eight out of 13 hamsters preferred the pine shavings, but this becomes 13 out of 13 if transfers (again, all of which were of pine shavings) are taken into account (P = 0.0001).

In the test involving aspen shavings versus wood pellets (Figure 2b), nine out of 13 (P = 0.1334) and 12 out of 13 (P = 0.0017) hamsters preferred the aspen shavings in the first and second trials, respectively. In both trials, the numbers become 13 out of 13 if transfers (all of which were of aspen shavings into the pellets cage) are taken into account.

Food was always placed evenly between the cages, be it at the beginning of the experiment or during replenishments. Food tended to remain present in all cages. However, hamsters sometimes built food piles in cage corners. For six of the eight trials conducted in these two experiments, there was no relationship between the position of the biggest food pile and nest position (χ^2 tests, *P* varies between 0.071 and 0.747). However, for the two trials that made the last cycle of the second experiment, the biggest food pile and the nest tended to be in the same cage (*P*=0.001 for each trial).

All hamsters used both of their wheels fairly evenly. In none of the eight trials conducted was there a significant difference between the number of wheel revolutions in the cage with the preferred bedding and the number in the less preferred cage (paired *t*-tests, *P* varies between 0.131 and 0.805, n = 13).

Discussion

In both experiments, results from the second trial with a particular pairing were consistent with those from the first. The expressed preferences are therefore replicable over a short period of time even when experience with other types of bedding material intervenes.

Preference was seldom absolute in the first trial. Most hamsters slept and built nests in both cages. Preference tended to be stronger in the second trial: a preference criterion of 75% would have yielded much the same results, in second trials, as did our less restrictive criterion of 50%. This does not mean, however, that the same preference would continue to be expressed for the rest of a hamster's life. Only experiments lasting over months could begin to address this question. No direct observations were made at night, so that bedding material preference can only be interpreted here in light of what hamsters do during the day, which is for the most part sleep. Given that hamsters in our laboratory spend most of their time running in wheels at night, the fact that both running wheels were used equally suggests a lack of bedding material preference related to night activities (see Ras *et al.* 2002 for a similar conclusion applied to rats).

In general, shavings were preferred over granules (though we did not test aspen shavings versus corn cob). One possible explanation for this result is that hamsters chose the most stable and most comfortable material to build nests for sleeping (see Rajendram et al. 1987, Ago et al. 2002). Granular material like pellets and corn cob cannot be piled high in a shape that surrounds the body of the sleeping hamster. Indeed, the few nests we observed in the cages with granular material were rather flat and small, in contrast to the cup-shaped nests made of shavings, a material that is softer and more pliable. The fact that only shavings were transferred between cages, always to be used as nest material in the new cage, supports the idea that hamsters chose bedding materials primarily for their quality for nest construction. Moreover, granular material (especially the round cylinders made by wood pellets, the least preferred of the materials we tested) can roll underfoot where the bedding layer has been thinned and may have made walking uncomfortable.

This issue raises the question of whether the preference for shavings would remain if material better suited for nest building (nesting material) were provided in addition to the bedding material. This is addressed in the next experiment.

Experiment II

Material and methods

Details were as in Experiment I. except that the choice apparatus was made of four cages linked by tunnels. The tunnels were of the same dimensions as before, but now formed a square with descending branches at each corner, each branch giving access to a cage. Each cage had food, water and a running wheel, though the running wheels this time were not connected to a computer and their revolutions were not tallied. Each cage contained a different bedding material (the same four as in the previous experiment), arranged in random order, and a partially torn sheet of paper towel as nesting material (see Van de Weerd et al. 1996, 1998). Litter was changed every 10 days, at which time new bedding and paper were provided in the same cages.

Fourteen new male hamsters (60 days old, from Charles River Canada) were each placed in a different apparatus. The nature of the bedding (cage) on which they were first deposited was systematically varied between individuals. For the next 31 days, the position (cage) of each hamster and its nest was noted daily. Notes were also made on a daily basis of the presence of food, faeces and urine in each cage. Running wheel activity was not tallied.

Results

On average (n = 14), hamsters were found in their favourite cage on 20.4 out of 31 days, and in their second favourite cage on 7.1 out of 31 days. At the group level, no bedding material was significantly preferred over another (Table 1). Together, the two shaving types were the first choice of only eight out

Table 1 Number of hamsters, out of 14, choosing a given material as their favourite or second favourite bedding when nesting material (paper) is provided

	Pine shavings	Aspen shavings	Corn cob	Wood pellets	χ^2 test
Favourite	2 (23.0)	6 (18.0)	2 (22.5)	4 (21.2)	χ ² =3.71, <i>P</i> >0.3
Second favourite	5 (7.0)	4 (6.25)	2 (8.0)	3 (8.0)	

Numbers in parentheses show the mean number of days, out of 31, on which the hamsters who selected the bedding type were found on that bedding

of 14 hamsters (P = 0.3953). Two of the 14 hamsters preferred the bedding material on which they had been placed at the beginning of the experiment, a result not different from random. Except for one hamster that slept for three days in a nest of pine shavings transferred onto wood pellets, there were no transfers of bedding material from one cage to another. Paper towels were used as nest material by all hamsters.

As in Experiment I, food remained present in all cages. Faeces were also present in all cages, but were more abundant in the cage where the nest was. Accumulation of urine tended to appear in one corner of the cage where the nest was.

Discussion

No significant preference for bedding type was expressed among the hamsters. Even within hamsters, preference was not absolute, as only about two-thirds of all days were spent on the favourite material. Thus there seems to be little preference between the bedding types if nesting material is provided. These results support the hypothesis that the preferences expressed in Experiment I was caused by the relative suitability and comfort of the bedding types as nest material.

Preference mostly for comfort raises the possibility that hamsters may choose bedding material that, though suitable in the short term for nest construction, may be suboptimal in the long term for health. In Experiment I, pine shavings were preferred by most hamsters, yet casual observations in our laboratory suggests that bleeding of the feet occurs more often when hamsters are housed on this bedding material. Maybe shaving pieces get wedged between the bars of the running wheels and cut the paws, or various compounds in pinewood soften the paws and make them more prone to cuts and abrasions. In the following experiment, we examined the effect of pine shavings, aspen shavings and corn cob on hamsters' paw condition, weight gain and running wheel activity over a period of 50 days.

Experiment III

Material and methods

Thirty new male hamsters, 60 days old, were purchased form Charles River Canada. Each was placed in a single cage (Nalgene F-size for rats). Each cage was equipped with a running wheel (F-size for rats) connected to a computer that tallied wheel revolutions daily. Water and food were replenished regularly as in the previous experiment. Ten hamsters were on pine shavings, 10 on aspen shavings and 10 on corn cob. The volume of each bedding material was 1 L. Litter was changed every 10 days over a period of 50 days.

Hamsters encounter wheels for the first time of their life when they arrive in our laboratory. They immediately start running at high daily levels, and scabs often appear on some of their feet. Every five days, the hamsters were placed in a small transparent box and their paws were examined. The total number of scabs on all four feet was noted. The severity of each scab was also noted, according to the following scale: 1 =small black dot, 2 = small healing white scab, 3 = red scab, 0.5 - 1.5 mm in diameter, 4 = redscab larger than 1.5 mm. An overall scab severity index was calculated for each hamster by adding up the severity indices of the scabs of all four paws. Every 10 days (at the time of litter change) the hamsters were weighed and the weight gain since the beginning of the experiment was calculated. The hamsters quickly got used to handling. Running wheel revolutions were tallied as in Experiment I, but equipment failure forced us to discard 20 days worth of data for all animals (the second and third 10-day blocks).

Repeated-measure ANOVAs (SPSS for Windows) were used to compare the three bedding treatments in terms of the total number of scabs on the hamsters' feet, overall scab severity, body weight gain since the beginning of the experiment and average daily wheel revolutions. The repeated measures were the data gathered every five days for scabs, the data gathered every 10 days for weight, and the average daily wheel revolutions for the 1st, 4th and 5th 10-day blocks. To allow for the possibility that the treatments might have affected hamsters in the same way in the short run, but differently in the long run, a one-way ANOVA was conducted only on the data gathered on the last day of the experiment (or the last 10-day block in the case of wheel running).

Paw condition and weight gain could be affected by the amount of running wheel activity. We therefore considered comparing the three bedding treatments in an ANCOVA that used running wheel activity as a co-variable, with all variables averaged for each animal over the 1st. 4th and 5th day block (the 2nd and 3rd block were discarded because of the lack of wheel running data due to the equipment failure mentioned above). However, only the pine shavings group showed the expected correlations (less weight gain and worse paw condition with higher running levels), the two other groups showing no significant correlations, and so an ANCOVA was not appropriate.

Results

The three bedding treatments did not significantly differ from one another in terms of either the number of paw scabs per animal (F = 1.172, P = 0.325), the overall scab severity (F = 1.188, P = 0.320), weight gain (F = 1.083, P = 0.353) and wheel running (F = 0.913, P = 0.413) over the 50-day period (Figure 3). Even if only the end of the experiment is considered, there is still no difference between the groups (scab number: F = 0.991, P = 0.384; scab severity: F = 0.573, P = 0.571; weight gain: F = 0.902, P = 0.417; wheel running: F = 2.818, P = 0.077; note that Figure 3 shows standard errors and not standard deviations).

Discussion

The lack of detected differences raises the question of whether the experiment lasted long enough for any possible effect to manifest itself. It must be pointed out that paw condition was improving for all three groups during the second part of the experiment (Figure 3), which suggests that



Figure 3 Evolution of various parameters (means + standard error, n = 10) in hamsters housed with different bedding materials over a 50-day period

any possible late effect would not be an extensive one. Weight gain and running activity also seemed normal for all three groups, and so any possible late decline would have to be precipitous to be worrisome from a welfare point of view. Nevertheless, we cannot discount the possibility of various long-term effects not detectable by our 50-day long experiment, and therefore our conclusion – that bedding material does not affect paw condition, weight gain and wheel running activity in hamsters – is of practical interest only in situations where hamsters are kept in captivity for a maximum of a few months.

As for all negative conclusions, considerations of statistical power must be addressed. Perhaps our sample size and statistical test were not sufficient to detect a small effect. If so, it must be pointed out that the tendency shown in Figure 3 is for a beneficial effect of pine shavings (more weight gain and better paw condition), a result that is consistent with the preference exhibited in Experiment I, but not with our original expectation based either on previous experience or on some reservations expressed in the pet care literature (e.g. Alderton 2002, Bucsis & Somerville 2002) about the suitability of resinous (cedar or pine) shavings as bedding material for hamsters. Any small effect that followed the trends of Figure 3 would therefore be difficult to interpret.

General discussion

Choice tests like those we used in this study only give relative preference between alternatives, so a non-preferred bedding material is not tantamount to a disliked or inappropriate material (Duncan 1992). Most hamsters spent at least some of their sleeping time on their least preferred material (Figures 1 and 2), indicating that none of the tested materials was strictly unacceptable.

In the absence of nesting material, pine shavings were the preferred bedding material. Pine shavings were also the cheapest of the four alternatives we tested. Over 50 days, pine shavings did not significantly affect paw condition and body weight relative to the other materials, but longer experiments with higher sample sizes are necessary to confirm this preliminary result. Such experiments should also test females as well as males, as in Reebs and St-Onge (2005).

The most straightforward conclusion from our study is that hamsters, in the short term, seem to prefer bedding material that is suitable for nest construction. When specific nesting material is provided, there appears to be little preference for one type of bedding material over another. Bedding materials may still differ in other ways than comfort, for example in terms of waste absorbency or their capacity to sustain bacteria, but any adverse effects this might cause is likely to be minimized, perhaps to negligible levels, by regular replacement of the litter. Thus, in standard conditions of animal care (i.e. regular litter replacement), and with nesting material being provided, we cannot as yet recommend one bedding material over another among the four types we tested.

Another conclusion that rises from our results is that hamsters exhibit a strong tendency towards nest building, even at room temperature. Nesting material should always be provided for this species, either in a form suited specifically to that use, or in the form of a bedding type that can double as nesting material, such as shavings.

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