Preferences of mice and rats for types of bedding material

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Summary

The type of bedding material has been reported to affect the environment in both the animal cage and animal room. It has an impact on the health and well-being of the animals and may cause biased experimental results. Requirements for bedding materials, particularly those regarding animal comfort are poorly supported by experimental data. In this study, various types of bedding material were evaluated using preference tests with mice and rats. It was found that beddings consisting of relatively small particles ($\leq 1.2 \times 1.6\,mm^2$) were generally avoided, whereas beddings consisting of large fibrous particles were preferred. The characteristics of the bedding materials were further investigated by scanning the size and shape of the particles, and by the assessment of ultrasound produced by the moving of the beddings. The results seem to indicate that size and manipulability are among the main determinants of the appreciation of bedding particles by laboratory mice and rats, and larger particles are preferred.

Keywords Bedding; environment; preference; behaviour; rat; mouse

Rodent beddings serve to absorb moisture from excrements and provide nesting material. The type of bedding material affects not only the microenvironment of the animal cage (Hirsjärvi & Väliaho 1985, Raynor *et al.* 1983), but also the environment of the animal room (Sakaguchi *et al.* 1989). Furthermore, the type of bedding may interact with experimental treatments and affect the outcome of certain experiments such as those on enzyme-inducing, cytotoxic and carcinogenic compounds and anaesthetics (Törrönen *et al.* 1989, Potgieter & Wilke 1992). To enhance comparability and reproducibility of results between experiments, the type of bedding should be standardized (Beynen 1991). The question then arises on how to define and select proper rodent bedding.

In order to sustain the health and well-being of laboratory animals and animal caretakers, and to minimize variability in experimental results, requirements for bedding materials have been listed (Kraft 1980, Wirth 1983, Weichbrod *et al.* 1986). These requirements include accommodating animal comfort, such as not being harmful, being nestable and allowing natural behaviour. However, it is not known which types of bedding meet these criteria.

The suitability of bedding for nesting by mice has been studied. Mulder (1975) reported that pregnant mice invariably selected aspen bedding from 10 different commercially available bedding materials. Odynets *et al.* (1991) using C57BL/6, BALB/c

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and wild mice also found that for nest building aspen bedding was the favourite of five different types of bedding material. These studies indicate that the origin of bedding material is an important determinant in the appraisal by the mice, but other properties such as particle size or dust content may be important as well. When offered a choice of various possibilities, rodents may be able to select the most suitable type of bedding or cage floor covering. On this assumption preference experiments were conducted with mice and rats to investigate their appraisal of different types of cage floor covering. In an attempt to elucidate the basis for the observed choice behaviours of the animals, selected properties of the beddings used were studied.

Materials and methods

Preference test systems

The test systems used for mice (Blom *et al.* 1992) and rats (Blom *et al.* 1995) have been validated and described in detail. Briefly, we used a multiple-choice housing system consisting of a central cage surrounded by either two or four test cages. The wire-topped central cage was made of transparent perspex and was lacking food and water. Macrolon type I (UNO Roestvaststaal, Zevenaar, The Netherlands) and type 375 (RUCO Metaalindustrie, Valkenswaard, The Netherlands) were used as test cages for mice and rats, respectively. The test cages were supplied with a known amount of food pellets (RMH-B®, Hope Farms, Woerden, The Netherlands) in the food hopper of the wire top and had a drinking bottle with a known amount of tap water. The type of floor covering differed between the test cages and between experiments. The central cage and test cages were connected by passages.

Each choice test started with the introduction of a single animal in the central cage of a test system with thoroughly cleaned cages and passages. During the test period of 48 h the animal could move freely from one cage to another. In the test system for mice, all passages between the cages were recorded photo-electrically. In selected tests, the behaviour of the C57BL/6JIco mouse was recorded on video. In the rat test system, passages were recorded by means of electronic balances which were placed under the test cages. Continuous turning of the preference test system during the testing of each mouse or turning its position between the testing of each rat was performed to cancel out any bias of choice behaviour related to the position of the preference test system within the experimental room. At the end of each test period, animal preference was assessed by calculating (relative) dwelling times per cage and occasionally analysing animal behaviour per cage.

Preference tests

Four experiments were conducted with mice and one with rats. Table 1 summarizes the types of floor covering tested. In the first series with mice, the choice behaviour was studied in a test system with four test cages. The floor of the test cages was covered either with the wire mesh, aspen wood chips, shredded filter paper or Hahnflock® H9 sawdust (Fig. 1, panel A). The perspex floor of the central cage had no covering throughout.

In the experiments with rats there were four test cages with different types of bedding: Hahnflock® H9, Lignocel® S8/15, Lignocel® 3/4 or Hahnflock® Tierwohl. All these beddings were manufactured of fir/spruce, but differed in particle size (Fig. 1, panel B). The floor of the central cage was covered with a wire mesh (stainless steel wire: rod diameter, 3.3 mm; mesh size, $40 \times 40\,mm^2$).

In three more experiments with mice, a test system with two test cages was used. The test cages were fitted with wire mesh versus wood chips; wire mesh versus Hahnflock® H9 sawdust; and wood chips versus Hahnflock® H9 sawdust. The floor of the central cage was again left without covering.

Animals and husbandry

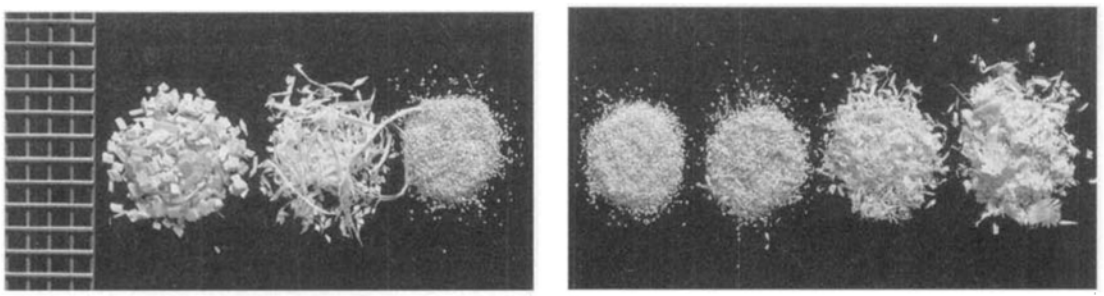
For the first experiment with mice, 20 female C57BL/6JIco and 20 female BALB/cBYJIco animals, aged 6 to 24 weeks, were used. Nine to 16 of these mice of each strain were used in the three experiments conducted later. Before and between the experiments, the mice were

Table 1 Types of floor covering material used in the test cages during the preference tests with mice and rats. (Fig. 1 shows the materials listed)

Floor covering material	Trade name	Type of particles	Size	Supplier
Wire mesh	not applicable	not applicable	rod diameter, 2 mm mesh size, 10 × 10 mm^2	UNO Roestvaststaal, Zevenaar, The Netherlands
Wood chips	Finn Tapvei aspen	chips	4 × 4 × 1 mm^3	Tapvei OY, Kaavi, Finland
Filter paper	Whatmann chromatographic paper type 3MM Chr.	shreds	approx. 2 × 5 mm^2	Boom, Meppel, The Netherlands (shreds were produced by cutting sheets of paper)
Sawdust	Hahnflock® H9	granulate	0.9 × 1.3 mm^2	Hahn & Co, Bredenbek-Kronsburg, Germany
Sawdust	Lignocel® S8/15	granulate	1.0 × 2.5 mm^2	Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany
Wood shavings	Lignocel® 3/4	fibres	2.0 × 7.0 mm^2	Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany
Wood shavings	Hahnflock® Tierwohl	fibres	2.0 × 4.5 mm^2	Hahn & Co, Bredenbek-Kronsburg, Germany

housed in groups of five animals in a housing system consisting of two wire-topped Macrolon type I cages connected by a passage. This housing system allowed the animals to become accustomed to moving through the passages of the test system. Both cages of each system were supplied with food pellets, water and sawdust bedding (Lignocel® S8/15). Stock and experimental rooms (conventional) had a controlled photoperiod (stock and experimental room: white light, approx. 200 lx at 1 m above the floor from 07:00–19:00 h, dark in stock room, and 700 nm, red light, approx. 5 lx at 1 m above the floor in the experimental room from 19:00–07:00 h), temperature (20–22 °C), relative humidity (50–60%) and ventilation (15 air changes per hour).

In the experiment with rats, 12 female and 12 male Wistar (Cpb:WU) animals, bred at the Laboratory Animals Centre, Wageningen, were used. The rats were tested at the age of 12 to 31 weeks; each rat was tested once. The animals had been born and housed in Macrolon type 375 cages with sawdust bedding (Lignocel® S8/15) until weaning. From weaning to the beginning of the experiment, the animals were housed in



A B

Fig 1 Types of cage floor covering used in the preference tests with mice (panel A (from left to right): wire mesh, FinnTapvei 4 × 1 ×1 aspen wood chips, shredded filter paper, Hahnflock® H9 sawdust) **and rats** (panel B (from left to right): Hahnflock® H9, Lignocel® S8/15, Lignocel® 3/4, Hahnflock® Tierwohl). Table 1 documents further characteristics of the floor coverings

groups of three in metal cages (type KS/4, RUCO Metaalindustrie) with wire mesh floors (stainless steel wire: rod diameter, 2 mm; mesh size, $10 \times 50\,mm^2$) and sawdust bedding (Lignocel® S8/15) at 70 mm underneath. The rats had free access to food pellets (RMH-B®) and tap water. Immediately before testing, each rat went through a pre-test (habituation) period of 48 h in the test system with uniform cages. Each test cage was fitted with a wire mesh floor (stainless steel wire: rod diameter, 2 mm; mesh size, $10 \times 30\,mm^2$) with sawdust bedding (Lignocel® S8/15) at 25 mm underneath. The animals were kept and tested in conventional rooms with controlled photoperiod (06:00–18:00 h: light, approx. 470 lx at 1 m above the floor), temperature (20–22 °C), relative humidity (50–70%) and ventilation (18 air changes per hour).

Characterization of bedding materials

The bedding materials were further characterized in an attempt to explain the observed choice behaviours. Actual particle size and shape, as well as dust content, was assessed with the use of a scanning electron microscope (Stereoscan, Cambridge Inc, Cambridge, United Kingdom). The bedding materials were also studied for ultrasound production when moved. For this purpose, 50 ml of bedding material was put in a 250 ml glass beaker and stirred with a glass rod (approx. 60 rotations min^{-1}). Ultrasound production was detected at 25 cm above the bedding material with a sensitive condenser microphone (Type 4135, Brüel & Kjaer, Nieuwegein, The Netherlands), preamplifier (Type 2633, Brüel & Kjaer), measuring amplifier (Type 2610, Brüel & Kjaer), and programmable dual filter (Type PDF3700 B, Difa Benelux BV, Breda, The Netherlands).

Statistical procedures

For statistical analyses, dwelling times (sec) per cage for the 48-h pre-test or test period were transformed into their logarithms. A Pearson correlation matrix for dwelling times of different cages was constructed to obtain information on competition between cages. Differences between mouse strains or sex of the rats were evaluated using univariate repeated measures analysis. Differences between floor covering and interactions of floor covering and either mouse strains or sex of rats or rat preferences during the pre-test period was evaluated using multivariate repeated measures analysis (Wilks' Lambda test). The level of significance was pre-set at $P<0.05$.

The multivariate repeated measures analysis only identifies overall significant differences between cages. To indicate which floor covering was preferred or avoided in the experiments using a test system with four test cages, a paired *t*-test was performed. In order to take into account the greater probability of a type I error due to multiple comparisons, the level of statistical significance was pre-set at $P<0.0083$ instead of $P<0.05$ (Bonferoni's adaptation).

Results

In the experiment with four different types of floor covering, the two inbred strains of mice showed an equal ($P_{strain}=0.406$) preference pattern. There was a statistically significant difference between the test cages ($P_{floor\ covering}<0.001$). As can be seen from Fig. 2 there was a significant preference for shredded filter paper when compared with wire mesh, aspen wood chips or Hahnflock® H9 sawdust. The mean relative dwelling times for the floor coverings other than shredded paper were similar. Mouse strain and preference did not interact ($P_{strain \times floor\ covering}=0.281$). The Pearson correlation matrix showed that there was competition between shredded filter paper and sawdust ($r=-0.621$), indicating that preference for shredded paper frequently accompanied avoidance of Hahnflock® H9 sawdust. A similar negative relationship was present between wire mesh and Hahnflock® H9 sawdust ($r=-0.530$).

'Resting' is the main component of the behaviour pattern that determines preference by analysing dwelling times per cage. Figure 3 illustrates that the preference for the shredded filter paper was mainly due to this

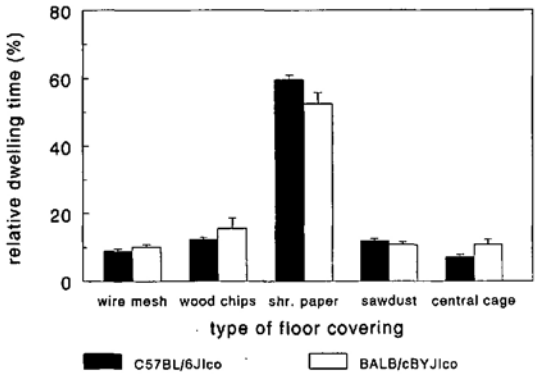


Fig 2 Distribution of relative times (means ± SEM; *n*=20) spent by female C57BL/6JIco and BALB/cBYJIco mice on four different types of cage floor covering. The preference test system used consisted of the central cage and four choices of floor coverings. The test period lasted 48 h, and thus by definition the total time spent in the five cages was also 48 h. Repeated measures analysis: P_{strain}=0.406; $P_{floor\ covering} < 0.001$; $P_{strain \times floor\ covering}$=0.281. Dwelling times for shredded paper differed significantly ($P < 0.001$, paired t-test) from those for the other three types of floor covering. Dwelling times for the wire mesh and wood chips were significantly different as well (P=0.0023, paired t-test)

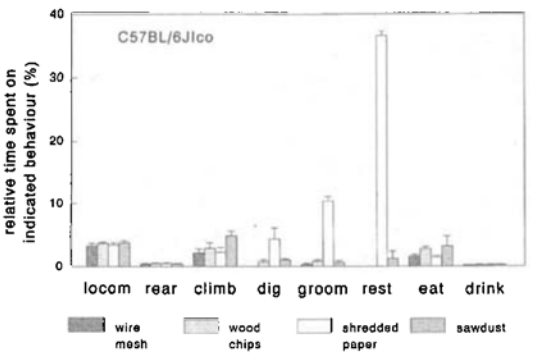


Fig 3 Distribution of times involved in eight behaviours (means ± SEM; *n*=3) as shown by female C57BL/6JIco mice on four different types of cage floor coverings offered in a preference test. The sum of the times spent on the eight behaviours on the four floor coverings equals 100% (=48 h)

behaviour. Also, the behaviour elements that are closely associated with resting, 'digging' and 'grooming', were seen most frequently in the cage fitted with shredded filter paper when compared with the three other types of floor covering. On average, other behaviour elements were found equally distributed over the test cages.

Table 2 shows the deposition of faeces and urine and the consumption of food and water in relation to the type of floor covering. Both strains deposited the lowest amounts of faeces and urine on the preferred floor covering, i.e. shredded filter paper. The consumption of food and water was similar for the four types of floor covering.

In the pre-test period of the preference tests with rats, the dwelling profile for the identical test cages for the females (27.4 ± 8.5, 12.6 ± 3.5, 15.8 ± 3.0 and 31.0 ± 5.2%; means ± SEM) and for the males (16.5 ± 2.5, 21.1 ± 4.7, 16.9 ± 3.5 and 33.0 ± 4.3%; means ± SEM) were similar. The effect of type of bedding, as shown in Fig. 4, was statistically significant. Also, the choice behaviour of males and females differed significantly. Both sexes avoided Hahnflock® H9 sawdust, but males tended to prefer Lignocel® 3/4 wood shavings and female rats showed a tendency of preference for Hahnflock® Tierwohl. Male rats spent more time in the central cage than females.

The types of floor covering offered to the mice were studied more closely in a test system with two test cages. To find whether there is a preference hierarchy for aspen wood chips, Hahnflock® H9 sawdust and wire mesh, these floor coverings were compared. The results show that both mice strains had similar preferences. Wood chips were preferred over wire mesh (Fig. 5A), and so was Hahnflock® H9 sawdust (Fig. 5B), while aspen wood chips were favoured over Hahnflock® H9 sawdust (Fig. 5C).

Figure 6 shows scanning electron micrographs of the bedding materials. The bedding materials that were favoured in the preference tests with mice and rats, whether shredded filter paper, wood chips or Lignocel® 3/4 wood shavings, consisted of relatively large, rough, fibrous particles. Of the bedding types that were relatively avoided in the tests, Hahnflock® H9 sawdust appeared to consist of uniform but relatively small particles. Hahnflock® Tierwohl wood shavings showed a wide range in particle size and shape and a large proportion of dust.

Table 2 Relative deposition of faeces and urine and relative consumption of food and water by female C57BL/6JIco and BALB/cBYJIco mice on four different types of floor covering in a preference test

C57BL/6JIco

	Type of floor covering			
	wire mesh	wood chips	shredded filter paper	sawdust (Hahnflock® H9)
Faeces (% of total boli)	19.6±1.9	27.6±1.9	16.1±2.1	35.0±2.1
Urine (mean urine score*)	1.6±0.1	1.7±0.1	1.1±0.1	2.0±0.0
Food (% of total g consumed)	22.2±2.4	23.0±1.9	26.6±3.0	28.2±2.8
Water (% of total ml consumed)	23.1±2.0	23.8±1.2	27.6±1.4	25.6±1.4

BALB/cBYJIco

	Type of floor covering			
	wire mesh	wood chips	shredded filter paper	sawdust (Hahnflock® H9)
Faeces (% of total boli)	28.6±2.2	27.8±2.3	10.3±0.9	31.3±1.8
Urine (mean urine score*)	1.7±0.1	1.9±0.1	1.1±0.1	2.0±0.0
Food (% of total g consumed)	26.8±2.8	21.6±1.9	22.1±2.4	29.5±2.5
Water (% of total ml consumed)	22.2±1.7	26.0±3.0	25.7±1.9	26.1±2.0

Results are expressed as means ± SEM for 20 mice per strain
*Urine was scored as: 1 dry; 2 moist; 3 wet; 4 soaking

To assess ultrasound production of the bedding materials when moved by the animals, the frequency and relative intensity of ultrasounds produced after stirring with a glass rod were measured. Ultrasound production was strongest with Hahnflock® Tierwohl wood shavings, whereas Hahnflock® H9 sawdust produced the fewest ultrasounds (data not shown). In general, ultrasound intensity increased with increasing particle size of the beddings tested. The highest levels of the ultrasounds were found in the lower frequency ranges (20–40 kHz).

Discussion

Figure 2 illustrates that shredded filter paper was so attractive to female laboratory mice that it masked differential preferences for wood chips, Hahnflock® H9 sawdust and a wire mesh floor. In the test system with two test cages, wood chips were preferred over sawdust and wire mesh. The preference patterns of the two inbred strains used were generally similar. Apart from relative dwelling times, the mice also discriminated between the types of floor covering with respect to defaecation and urination. The mice tended to deposit less faeces and urine in the cage with shredded filter paper, i.e. the cage where they spent most time on resting. This suggests that to a certain extent mice actively keep their nesting area clean from excreta. Under practical conditions, multiple cage compartments usually cannot be offered. When shredded paper is given as the sole bedding material it may become wet quickly. Since the absorbing potency of sawdust bedding exceeds that of shredded filter paper, a

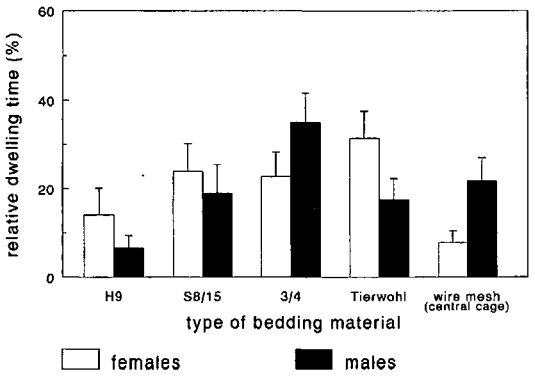


Fig 4 Distribution of relative dwelling times (means ± SEM; n=12 per sex) during the test period of 48 h for male and female Cpb:WU rats in a preference test system with the central cage and four test cages fitted with different types of bedding material. Repeated measures analysis: P_{sex}=0.004; $P_{bedding}$=0.010; $P_{bedding \times sex}$=0.173. Dwelling times for Hahnflock® H9 sawdust differed significantly (P=0.0015 to 0.0025, paired t-test) from those for the other three test cages

combination of both bedding materials may be favourable when animals are housed in a single cage.

In the preference tests with rats, data on faeces and urine production were not collected. It was however apparent that at the end of the test periods more faecal boli were present in the central cage with a wire mesh floor than in the test cages with various types of bedding. Possibly, rats also prefer a clean living area.

Both mice and rats appeared to avoid bedding material with relatively small particles (Hahnflock® H9 sawdust) and preferred beddings consisting of large particles (aspen wood chips) or fibres (Lignocel® 3/4 wood shavings, Hahnflock® Tierwohl wood shavings, shredded filter paper). Bedding materials consisting of relatively small particles are unsuitable for nest building (Hämäläinen & Tirkkonen 1991). The observed preferences for bedding materials may relate to their suitability as nesting material. Analysis of mouse behaviour revealed that digging, i.e. manipulation of bedding, and resting in a nest were performed most on the preferred bedding. The preference of the mice for shredded filter paper is in accordance with the results of Nolen and Alexander (1966). In a limited number of additional choice tests with four test cages we offered mice a cage with a sheet of filter paper fixed to the floor instead of shredded filter paper. The filter paper was now only preferred when the animals managed to remove and tear the sheet (Fig. 7). If they did not succeed in doing so, the cage with filter paper floor covering was avoided. Apparently, the structure of filter paper that has been shredded may determine preference of the mice.

Small bedding particles and especially dust (particles smaller than 300 µm, Wirth 1983) can be irritating or damaging when inhaled (Sakaguchi *et al.* 1989, Thigpen *et al.* 1989). Such particles also irritate the vaginal mucosa (Plank & Irwin 1966) or cause preputial infections (Van Herck, personal communication). In this light it is plausible that Hahnflock® H9 sawdust is avoided when compared with the other bedding materials tested. Nevertheless, the female rats tended to prefer Hahnflock® Tierwohl wood shavings as bedding material, despite its high proportion of dust.

Rodents are very sensitive to ultrasound (Ralls 1967, Sales & Milligan 1992). We assumed that moving of bedding materials by rodents may produce ultrasonic sounds that can either be irritating or pleasurable. The bedding materials tested were found to produce ultrasounds upon stirring, the highest intensity being caused by Hahnflock® Tierwohl wood shavings. This characteristic of Hahnflock® Tierwohl could have contributed to the relatively high degree of appraisal by the female rats. The same may be true for the preference towards aspen wood chips (Finn Tapvei) when compared with Hahnflock® H9 sawdust, as displayed by the mice. Thus, relative preference and/or avoidance of a given bedding material may be associated with ultrasound production.

The results of this study indicate that size and/or shape might be a major determinant in the appreciation of bedding particles by laboratory mice and rats. Beddings consisting of large fibres seem to be preferred. It should however be emphasized that the present data cannot be interpreted unequivocally to show

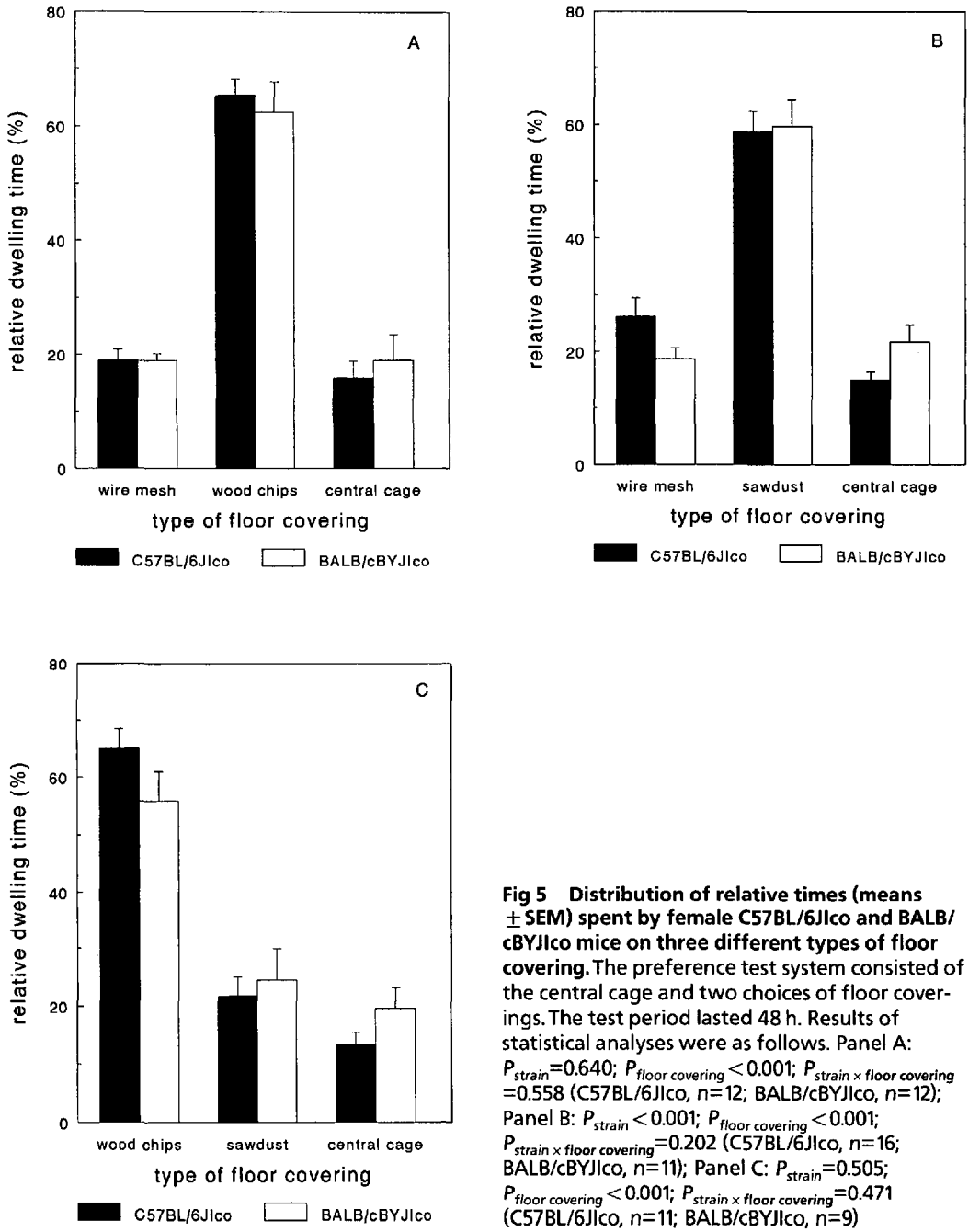


Fig 5 Distribution of relative times (means ± SEM) spent by female C57BL/6JIco and BALB/cBYJIco mice on three different types of floor covering. The preference test system consisted of the central cage and two choices of floor coverings. The test period lasted 48 h. Results of statistical analyses were as follows. Panel A: $P_{strain}=0.640$; $P_{floor\ covering}<0.001$; $P_{strain \times floor\ covering}=0.558$ (C57BL/6JIco, $n=12$; BALB/cBYJIco, $n=12$); Panel B: $P_{strain}<0.001$; $P_{floor\ covering}<0.001$; $P_{strain \times floor\ covering}=0.202$ (C57BL/6JIco, $n=16$; BALB/cBYJIco, $n=11$); Panel C: $P_{strain}=0.505$; $P_{floor\ covering}<0.001$; $P_{strain \times floor\ covering}=0.471$ (C57BL/6JIco, $n=11$; BALB/cBYJIco, $n=9$)

the influence of bedding particle size and shape. The bedding materials tested were not all made from the same starting material. Thus, apart from size and shape of the bedding particles, other characteristics may also have played a role in determining the observed preferences. Further, animals' characteristics such as strain, age, sex and reproductive condition may also determine the preference for bedding materials.

The results of preference tests, provided they are interpreted with care and reason,

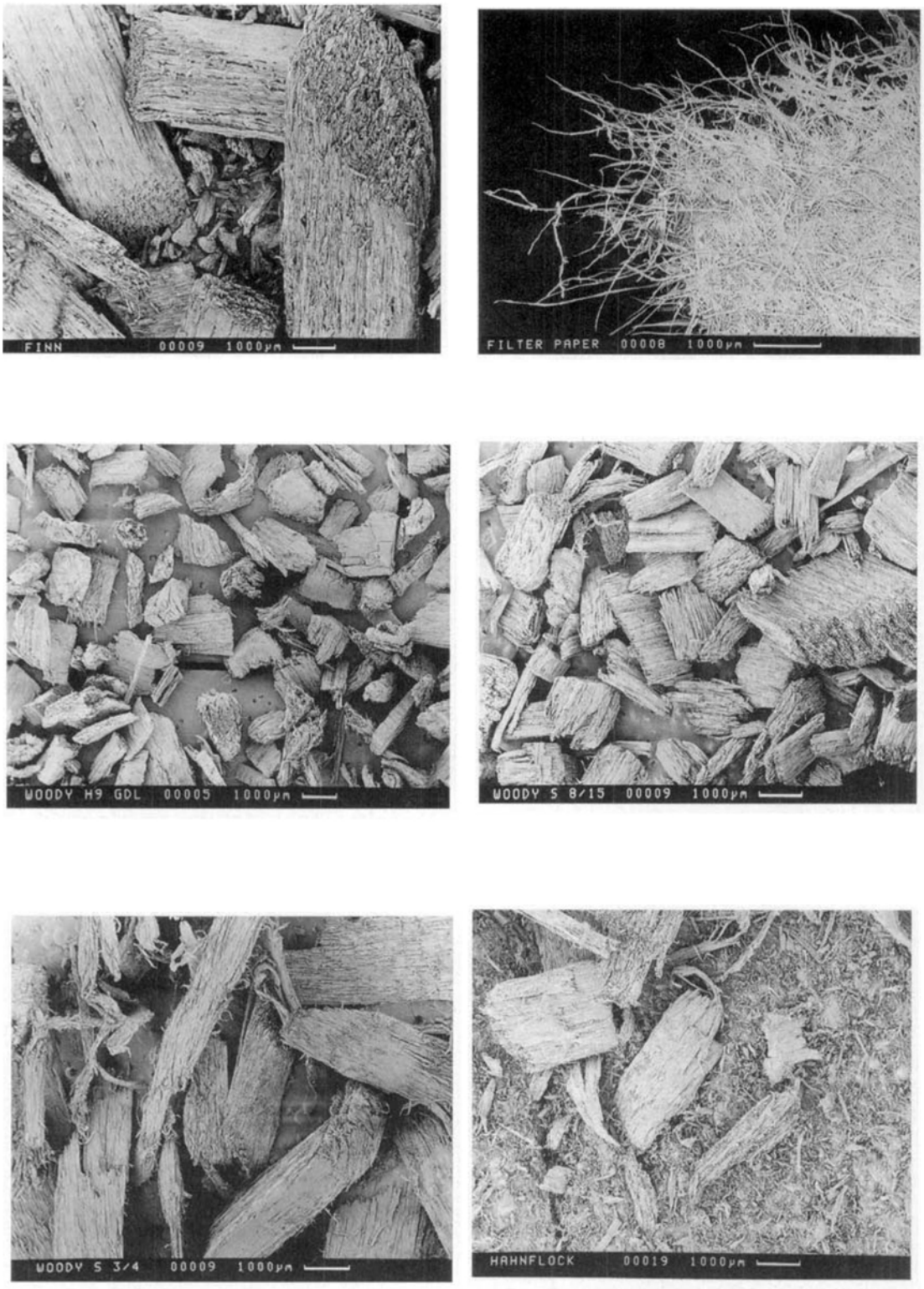


Fig 6 Scanning electron micrographs of bedding materials studied in preference tests with mice and rats. The photographs have approximately identical magnification: the bar indicates 1 mm

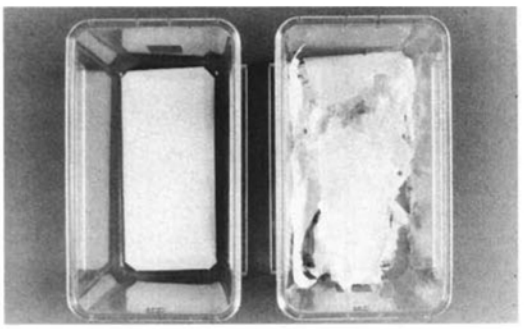


Fig 7 Illustration of a test cage with a sheet of filter paper fixed to the floor as used in a preference test system for mice consisting of the central cage and four test cages with different types of cage flooring (cf. Table 1). The test cage with the filter paper was either avoided (left) or preferred (right) depending on the animal being successful in creating a nest by tearing the sheet of paper

may contribute to the adequate adaptation of existing guidelines and practices on housing laboratory animals. Promoting the adaptation of laboratory animal housing systems towards the animals' needs may be conducive to animal welfare but does not necessarily sustain scientific interests. The use of preferred bedding material so as to improve the animals' well-being seems appropriate, but it should be realized that certain experiments require specific cage floor coverings, such as wire mesh bottoms, that are not compatible with the animals' preference. Thus, the introduction of changes should always be considered along with their consequences for the experiment.

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