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Preferences for Paper Bedding Material of the Laboratory Mice

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Abstract: In order to identify indicators of the preferences for bedding materials, the paper bedding material preferences of laboratory mice were investigated in the present study. Four cages, each containing a different structure of paper bedding material were connected to allow free access to each cage. The preferences for paper bedding materials of laboratory mice were judged by the differences in the length of stay and sleep in each cage. The mice preferred the bedding material that allowed them to easily hide and build nests and was soft. We conclude that the comfort and well-being of laboratory mice can be increased through the appropriate selection of bedding material.

Key words: bedding material, nest building, preferences

Among small laboratory animals, bedding material, in addition to cage size and shape, is an important environmental factor for sustained well-being. In Japan, electric wood shavings (hereafter called wood chips) are generally used as bedding material. The recent increased interest in protecting forest resources and the environment suggest the need for an alternative. Artificial bedding materials as substitutes for wood chips, namely paper bedding materials made from wood pulps, are being increasingly used in Japan and U.S.A. The ammonia concentration in cages, the amount of dust produced, and the effects of bedding materials on animals' organs and blood has been compared between wood chips and paper bedding materials [2, 4]. Some reports have compared these materials from an animal welfare point of view [1, 5, 6]. In the present study,

preference tests for the four common paper bedding materials for mice were conducted.

The experiments were carried out with 10 male ICR mice (CLEA Japan, Inc, Tokyo), which were bred in cages with wood chips for four weeks. The animals were transported to our institute at four weeks of age. After being acclimatized for two weeks in cages with autoclaved wood chips at our institute, the mice used for the experiments were six weeks of age. The artificial light/dark cycle in the animal room was 12:12 with lights on at 08:00 and off at 20:00. The room temperature was $24 \pm 2^\circ\text{C}$ and relative humidity was 50–60%. All mice had access to autoclaved food pellets (MF, Oriental Yeast, Co., Ltd. Tokyo Japan) and tap water *ad libitum*.

Four kinds of bedding materials sold at stores (Table

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

Trade name	Raw material	Type of particles	Size	Supplier	Manufacturer
ALPHA-dri	Virgin pulp 100%	Chip	approx. 5 × 4 × 1.5 mm	TOYOTA TSUSHO Co., LTD. Japan	Shepherd Specialty Papers, Inc. U.S.A.
CARE FEEAZ	Paper diaper waste	Fibrous	3 × 50 mm	HAMRI Co., LTD. Japan	HAMRI Co., LTD. Japan
Care FRESH	Tissue papers waste	Flatness	6 × 10–15 mm	NIKI-SHOJI Co. Japan	ABSORPTION CORP. U.S.A.
Paper clean	Recycled milk pack	Roll	diameter: 4 mm length: 10 mm	Japan SLC, Inc. Japan	Japan SLC, Inc. Japan

Fig. 1. Macroscopic appearance of four kinds of bedding materials.

1, Fig. 1) were used after sterilization via autoclaving.

Polymethylpentene cages for mice (190 × 260 × 125 mm) were used. Four cages were connected to a central cage via cylindrical passages (diameter 40 mm) to enable mice free access to each cage (Fig. 2). A different bedding material (volume 500 ml; ALPHA-dri: 100 g, CARE FEEAZ: 25 g, Care FRESH: 45 g, and Paper clean: 71 g) was placed in each of the outer cages and each mouse was initially placed into the central cage which had metal drainboards. In this experiment, we tested whether the mice's behavior patterns were able to become indicators of preferences. We made video recordings of the mice's behavior in the video recording. During the dark periods, illumination was provided by a dim red light to enable video recording. We observed which cages the mice relaxed in or slept in, and using a stopwatch (SEIKO system S129) measured the length of stay in each cage and the number of times the

mice crossed into other cages. Water bottles and food were put only in the central cage. The behavior patterns of 10 mice were observed for 8 hr (10:00–18:00) during the light period and 4 hr (23:00–3:00) during the dark period. This observation time zone was set up referring to the experimental result of Harri M. *et al.* [3].

The mice repeatedly visited each cage many times after the experiment began, and repeated various behaviors, i.e. searching, digging holes, standing up, hanging, and jumping. Following this, the mice generally became composed, and seemed to decide where to rest some time after 24 hr. Therefore, for this experiment, we observed their behavior patterns after 48 hr, when they had become more stable.

Table 2 shows the total length of stay according to time zone and each behavior pattern in each cage, during the light and dark periods. During the light and the dark period, the length of stay was largest in CARE FEEAZ and most of this time was spent sleeping. During the dark period, the length of stay was large in the central cage, where most of the time was spent feeding.

Table 3 shows the number of times the mice crossed between cages during the light and dark period. The number of crossings were mostly to the central cage, and a big difference wasn't recognized between the other cages. The number of crossings for around one/hour in the night was about 3.6 times that of the day.

Based on the results shown in Table 2, Figs. 3 and 4 were constructed.

Figure 3 shows the behavior patterns during the light and dark periods. The behavior patterns were divided into five main types (Acting: hanging, jumping and digging; Resting: grooming, face-washing and crouching; Nesting: gathering bedding material in the mouth; Sleeping: closing eyes and rounding the body; and Feeding:

Fig. 2. Experimental cages are connected to the central cage with cylindrical passages which have a diameter of 40 mm, so that mice can cross to each cage freely.

Table 2. Total length of stay with time zone and each behavior pattern in each cage (mean $\pm$ SEM; n=10)

	ALPHA-dri (seconds)	CARE FEEAZ (seconds)	Care FRESH (seconds)	Paper clean (seconds)	Central Cage (seconds)	(%)
10:00–11:00	267 $\pm$ 82	2359 $\pm$ 339	298 $\pm$ 94	119 $\pm$ 28	557 $\pm$ 206	
11:00–12:00	63 $\pm$ 44	3245 $\pm$ 828	43 $\pm$ 27	83 $\pm$ 57	166 $\pm$ 23	
12:00–13:00	23 $\pm$ 11	3315 $\pm$ 147	72 $\pm$ 57	38 $\pm$ 22	152 $\pm$ 89	
13:00–14:00	62 $\pm$ 32	3225 $\pm$ 175	59 $\pm$ 34	81 $\pm$ 38	173 $\pm$ 111	
14:00–15:00	16 $\pm$ 11	3451 $\pm$ 117	64 $\pm$ 61	41 $\pm$ 36	28 $\pm$ 20	
15:00–16:00	38 $\pm$ 20	3481 $\pm$ 44	19 $\pm$ 12	13 $\pm$ 7	49 $\pm$ 27	
16:00–17:00	50 $\pm$ 34	3366 $\pm$ 120	39 $\pm$ 28	36 $\pm$ 18	109 $\pm$ 59	
17:00–18:00	79 $\pm$ 41	3180 $\pm$ 235	46 $\pm$ 22	21 $\pm$ 9	274 $\pm$ 171	
10:00–18:00						
Acting	598 $\pm$ 97	476 $\pm$ 114	585 $\pm$ 100	432 $\pm$ 67	802 $\pm$ 163	10.1 $\pm$ 1.2
Resting	0	264 $\pm$ 198	55 $\pm$ 55	0	45 $\pm$ 45	1.4 $\pm$ 0.7
Nesting	0	2935 $\pm$ 608	0	0	0	10 $\pm$ 2.1
Sleeping	0	21947 $\pm$ 1019	0	0	0	76.2 $\pm$ 3.4
Feeding	0	0	0	0	661 $\pm$ 129	2.3 $\pm$ 0.5
Total staying	598 $\pm$ 97	25622 $\pm$ 379	640 $\pm$ 100	432 $\pm$ 67	1508 $\pm$ 302	
Frequency (%)	2 $\pm$ 0.3	89 $\pm$ 1.3	2 $\pm$ 0.3	2 $\pm$ 0.3	5 $\pm$ 0.9	
23:00–24:00	198 $\pm$ 53	2226 $\pm$ 374	232 $\pm$ 84	114 $\pm$ 33	830 $\pm$ 284	
24:00–1:00	271 $\pm$ 126	2462 $\pm$ 411	194 $\pm$ 88	66 $\pm$ 25	607 $\pm$ 160	
1:00–2:00	536 $\pm$ 108	1106 $\pm$ 429	372 $\pm$ 117	284 $\pm$ 53	1302 $\pm$ 71	
2:00–3:00	298 $\pm$ 58	1609 $\pm$ 344	663 $\pm$ 146	162 $\pm$ 54	868 $\pm$ 163	
23:00–3:00						
Acting	1226 $\pm$ 152	737 $\pm$ 183	1422 $\pm$ 261	626 $\pm$ 86	1368 $\pm$ 196	37.5 $\pm$ 2.5
Resting	77 $\pm$ 60	623 $\pm$ 231	39 $\pm$ 30	0	0	5.2 $\pm$ 1.5
Nesting	0	701 $\pm$ 143	0	0	0	4.8 $\pm$ 0.9
Sleeping	0	5342 $\pm$ 598	0	0	0	36.8 $\pm$ 4.1
Feeding	0	0	0	0	2239 $\pm$ 401	15.7 $\pm$ 2.8
Total staving	1303 $\pm$ 152	7403 $\pm$ 552	1461 $\pm$ 267	626 $\pm$ 86	3607 $\pm$ 517	
Frequency (%)	9 $\pm$ 0.9	52 $\pm$ 3.7	10 $\pm$ 1.9	4 $\pm$ 0.6	25 $\pm$ 3.5	

Table 3. Number of crossings to each cage (mean $\pm$ SEM; n=10)

	ALPHA-dri	CARE FEEAZ	Care FRESH	Paper clean	Central cage	Total	Average/1 hr
10:00–18:00	16 $\pm$ 2.6	24 $\pm$ 2.5	15 $\pm$ 1.6	14 $\pm$ 2.2	34 $\pm$ 4.8	103 $\pm$ 12.6	12.9
23:00–3:00	36 $\pm$ 1.9	29 $\pm$ 2.8	32 $\pm$ 2.5	26 $\pm$ 2.2	62 $\pm$ 2.7	187 $\pm$ 6.6	46.8

Fig. 3. Results of behavioral observation from the preference test. Mean relative spending time with indicated behavior during light and dark periods. In light periods, sleeping differed significantly ($p < 0.001$) from the other behaviors. In dark periods, acting and sleeping differed significantly ($p < 0.001$) from the other behaviors. **Fig. 4.** Mean relative dwelling times per cage for light and dark periods. Dwelling times for CARE FEEAZ differed significantly ($p < 0.001$; paired t-test) from the other cages.

eating and drinking). During the light period, the mice mostly slept in the cage and they also showed the patterns of nesting and grooming. On the other hand, during the dark period, they almost always expressed the patterns of acting and sleeping.

Figure 4 shows the length of staying time in each cage during the light and dark periods. In both periods, the mice mostly stayed in the CARE FEEAZ cage and they almost always expressed the pattern of sleeping. Following the CARE FEEAZ cage, the mice spent a lot

of time in the central cage, especially in the dark period and they showed the feeding pattern there. It has been reported that the bedding consisting of large fibrous particles is preferred compared to the small ones [1], and that the shape of the nesting material determined the nestability [5, 6]. The purpose of present study was to identify the indicators of mice's preferences for paper bedding materials. CARE FEEAZ is made from paper diaper waste and has a large fibrous shape (length; 50 mm) in addition to having the softest feel compared to the other materials, and CARE FEEAZ seemed to be the easiest material for mice to build their nests. These facts suggest that the shape of the bedding material is an indicator of mouse's preference, followed by smell, touch and material.

We are convinced that rearing with preferred bed-

ding material for nesting and resting would contribute to the well-being of laboratory mice.

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