

Evaluation of Bedding and Nesting Materials for Laboratory Mice by Preference Tests

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Abstract: Bedding and nesting materials can improve the health and environmental welfare of laboratory mice. This study was carried out to examine which items are actually preferred by mice. Two series of studies were performed on four types of floor-covering materials (Wood-shavings (Clean-chip™), Cloth (Agrebe™), Recycled-paper (Paper-clean™), Paper (Care-feeaz™)), and on four types of nesting materials (Recycled-paper (Shepherd-shack™), Cloth (Agrebe™), Wood (Wood-cylinder), and Polycarbonate (Mouse-igloo™)). Preference of bedding materials was judged by the time length of staying in a cage. The results indicate that mice stayed in the cloth material (Agrebe™) longer than in other bedding materials (light $51.1 \pm 5.3\%$, dark $51.5 \pm 2.6\%$). In the second experiment, the duration of stay in Agrebe™ was significantly longer than that in the other nesting materials in the light phase ($70.9 \pm 2.4\%$). In the dark phase, staying time both in Agrebe™ and Shepherd-shack™ were significantly longer. These data suggest that cloth bedding and nesting is recommended for the environmental enrichment of laboratory mice.

Key words: cloth bedding, environmental enrichment, mice, preference test

Introduction

Bedding material is one of the most important environmental factors for laboratory mice [19]. It is possible that a difference in bedding materials influences not only the animal's health and welfare but also experimental results [8, 13].

To date, wood chips, paper and others have been

used as bedding materials for laboratory mice. Many studies have tried to clarify what types of materials and structures are better as the bedding material for laboratory rodents [2, 4, 9, 16]. However, the increasing interest in protecting the environment and natural resources requires alternatives.

Recently, a cloth bedding material (Agrebe™) was developed for laboratory animals [7]. Agrebe™ has

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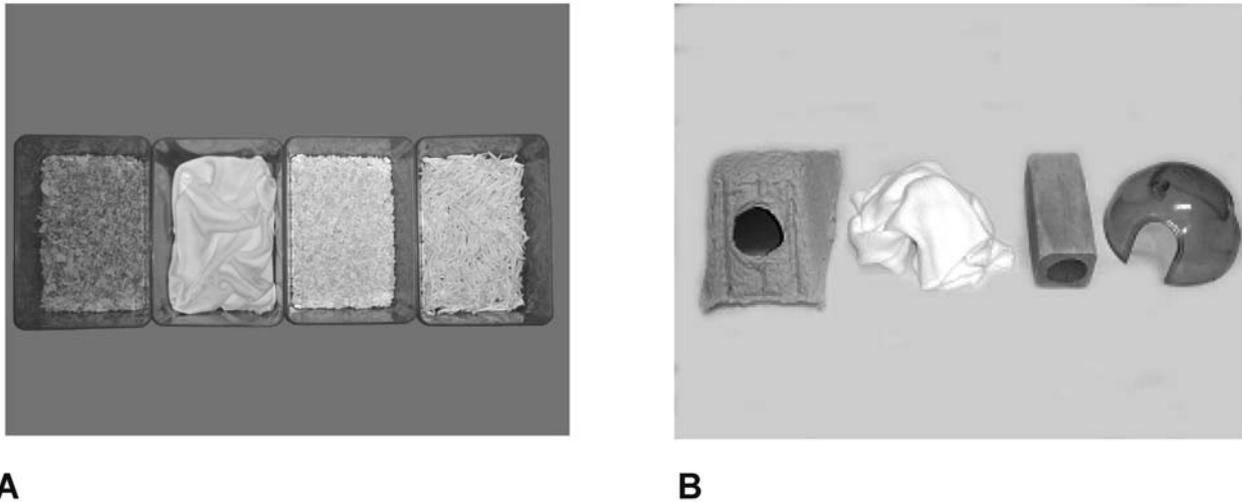


Fig. 1. Types of cage floor-covering and nesting materials used in the preference tests with mice. Panel A (from left to right): Clean-chip™ (CC), Agrebe™ (AG), Paper-clean™ (PC), Care-feeaz™ (CF). Panel B (from left to right): Shepherd-shack™ (SS), Agrebe™ (AG), Wood-cylinder (WC), Mouse-igloo™ (MI). Table 1 documents further characteristics of these materials.

the characteristic of adsorbing ammonia, and it is reusable after washing [6]. This may have several advantages in terms of animal welfare as well as the preservation of natural resources used as the bedding material for experimental animals. In the present study, preference tests were performed with this new bedding material and others. The preference tests were conducted to directly compare four different floor-covering materials and four different nesting materials for laboratory mice.

Materials and Methods

Animals and environment

Fifteen male ICR mice (Japan SLC Inc., Shizuoka), 10 weeks old, were used. Before the experiments, the mice were individually housed in plastic cages (175 × 245 × 125 mm, Japan Clea Inc., Tokyo) with a wire floor on the bottom of the cages. All animals were kept in an animal room for 2 weeks before the experiments. The room was kept at a constant temperature (23 ± 2°C), humidity (55 ± 10%) and light-dark cycle (lights on at 07:00 and off at 19:00). Animals were fed on a plain commercial diet (NMF, Oriental Yeast Co. Ltd., Tokyo) and tap water *ad libitum*. All experimental procedures were approved by the local committee for animal research of Shimane University School of Medicine.

Floor-covering materials and nesting materials

The preference tests were carried out in a test system with four cages. Two series of tests were performed using either four different types of floor-covering materials (Fig. 1A) or four different types of nesting materials (Fig. 1B). Table 1 shows further characteristics of these materials. All materials used in the experiments were sterilized by autoclaving.

Test systems

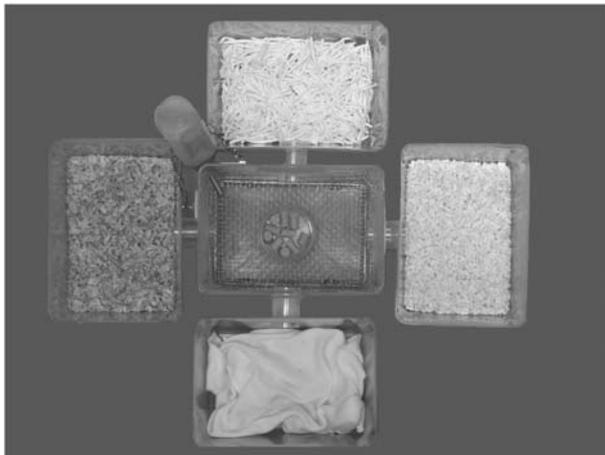
Preference was assessed by using the test system described previously [1]. The multiple-choice housing system consisted of a central cage (grid floor) surrounded by four test cages (plastic floor) (Fig. 2). The central cage and the four test cages were connected by passages. Feeder and water bottles were set in the central cage. Each test was started by introducing a single animal into the central cage. The animal could move freely from one cage to another. Animal behavior was monitored with a digital video camera (GR-DVX7, Victor Inc., Tokyo) under infra-red illumination every 5 min. After 24 h observation including light and dark phases (12 h each), the cages were cleaned and disinfected with alcohol before the next test.

Statistical analysis

Data are expressed as means ± standard errors of the mean (SEM). Differences were tested with analysis of

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

Material	Trade name	Size (mm)	Supplier
Wood shavings	Clean-chip (CC)	15.0 × 22.0 × 1.0	Shimizu, Inc., Japan
Cloth	Agrebe (AG)	400.0 × 400.0 × 2.0	Globe, Inc., Japan
Recycled-paper	Paper-clean (PC)	4.0 × 4.0 × 10.0	Japan SLC, Inc., Japan
Paper	Care-feeaz (CF)	2.0 × 2.0 × 70.0	HAMRI Co., LTD., Japan
Recycled-paper	Shepherd Shack (SS)	146.0 × 89.0 × 64.0 one small hole (φ 40)	Shepherd Specialty Papers, USA
Cloth	Agrebe (AG)	200.0 × 200.0 × 2.0	Globe, Inc., Japan
Wood	Wood-cylinder (WC)	40.0 × 40.0 × 100.0 circular hole (φ 40)	Japan CLEA, Inc., Japan
Polycarbonate	Mouse-Igloo (MI)	Diameter 10.0 × 60.0, hole (30.0 × 40.0) was three cut into the dome	Bio-Serv., USA

**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.

variance (ANOVA) and the Scheffe post-hoc test. Analyses were performed using Stat View (SAS Institute Inc., North Carolina, USA). $P < 0.05$ was considered significant.

Results

Experiment 1

In Fig. 3, the relative duration of stay of mice is shown for the central cage and the cages with the four types of floor-covering materials. Mice stayed significantly longer in the cage with Agrebe™ under both light ($51.1 \pm 5.3\%$) and dark ($51.5 \pm 2.6\%$) phases.

Experiment 2

Figure 4 shows the relative durations of stay in the wire central cage and in the four cages with different nesting materials. Mice stayed in the cage with Agrebe™ significantly longer during the light phase ($70.9 \pm 2.4\%$). In the dark phase, the relative duration both in Agrebe™ and Shepherd-shack were significantly longer. The values were $41.3 \pm 9.6\%$ and $41.9 \pm 12.9\%$, respectively.

Discussion

Bedding materials are an important factor for laboratory animals' daily behavior, such as playing, sleeping, nesting, eating and breeding [10, 20, 21]. Especially, mice are nocturnal animals that often prefer hiding and sleeping in dark places during the daytime [1, 3]. In addition, the shape of the nesting material determined the nest-building [12, 17], and the preferred nesting material provided shelter from overexposure to light [5, 11]. It has been recommended that preference testing is carried out over at least a 24 h cycle [2, 13], to ensure that differences in preference during the dark and the light periods are observed.

In this experiment, mice had a strong preference for the cloth bedding materials compared to the other materials. Actually, mice were observed resting or sleeping in the cloth bedding materials during the light period. During the dark period, mice were seen crawling and hiding within the cloth bedding material. This suggests that Agrebe™'s superior flexibility, hygroscopicity and

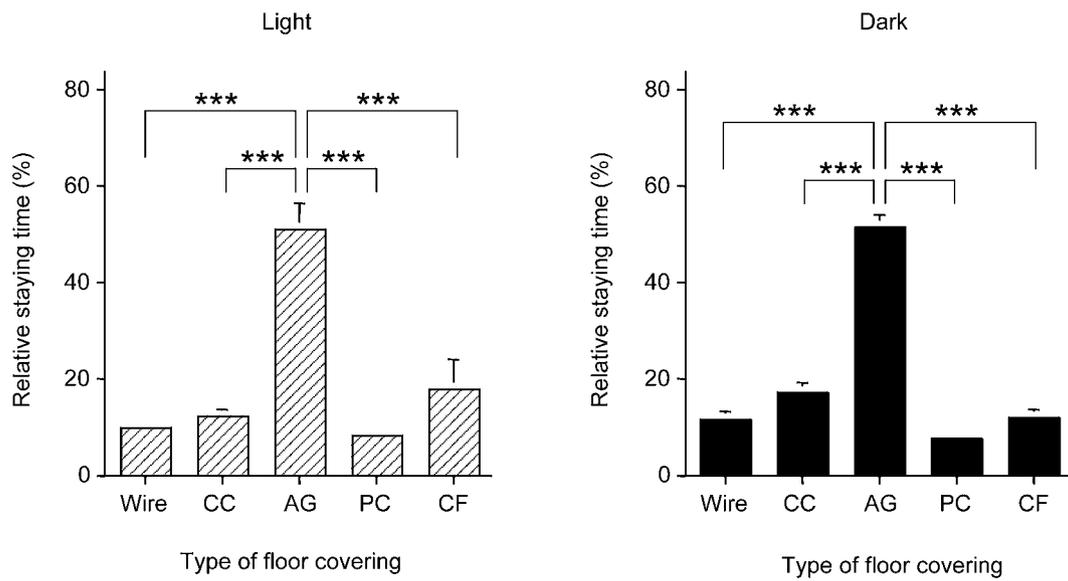


Fig. 3. Relative durations of stay of male ICR mice ($n=10$) in cages with four types of bedding materials during 24 h (light: 07:00–19:00, dark: 19:00–07:00). Central cage (wire), Clean-chip (CC), Agrebe™ (AG), Paper-clean™ (PC), Care-feeaz™ (CF). Results are expressed as the mean \pm SEM, Significant differences from central cage (wire) bar *** $P<0.001$.

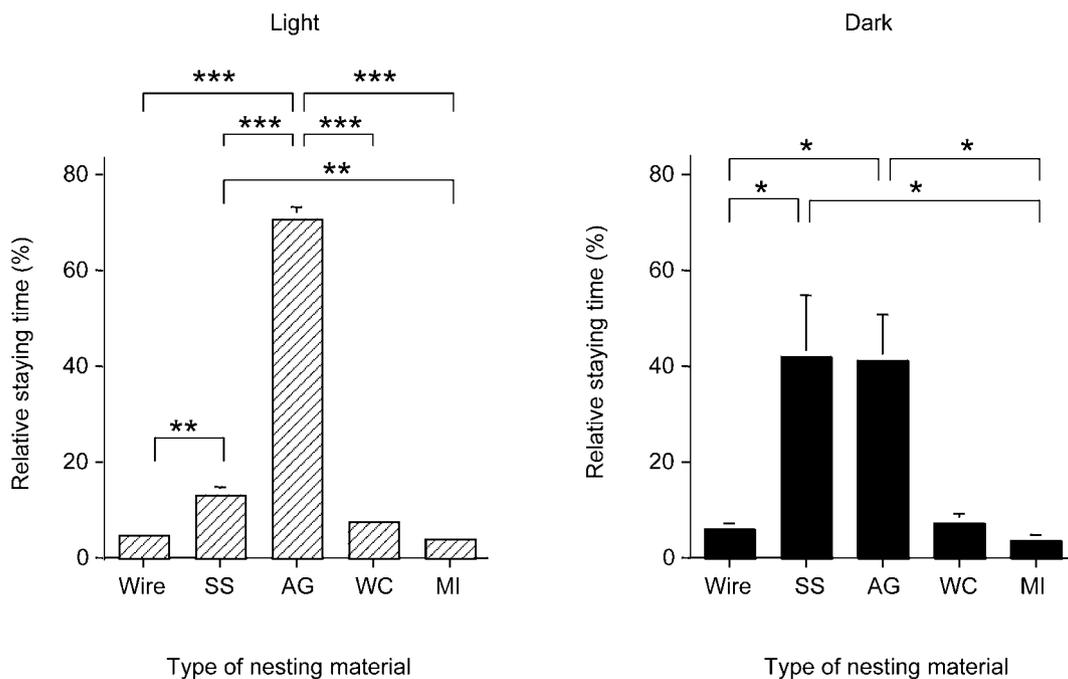


Fig. 4. Relative durations of stay of male ICR mice ($n=5$) in wire-cages with four types of nesting materials during 24 h (light: 07:00–19:00, dark: 19:00–07:00). Central cage (wire), Shepherd-shack™ (SS), Agrebe™ (AG), Wood-cylinder (WC), Mouse-igloo™ (MI). Results are expressed as the mean \pm SEM, Significant differences from central cage (wire) bar * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

heat-retaining properties might be preferred by mice to other materials.

Regarding comfort of the living environment, a bedding material is also assessed by the occurrence of ammonia and dust [22]. In our previous study, we reported that the ammonia concentration in Agrebe™ cages was 14 ± 1 ppm after 7 days of bedding exchange, whereas the concentration in paper bedding (paper-clean™) was 46 ± 18 ppm [7]. Moreover, the number of adhesion bacterium on the rack was significantly lower in the Agrebe™ group than in the paper bedding group. Similarly, Iwaki *et al.* [6] indicated that the amount of dust in an Agrebe™ group was less than in a paper bedding group. These results indicate that the cloth bedding is also excellent from the aspect of keeping the environment clean as well as being preferred by mice.

Moreover, the preference test of nesting materials showed that the mice preferred staying in the cloth material. In the present study, mice preferred Agrebe™ in the light period, and although almost the same staying time was recorded with Shepherd-shack™, mice also preferred Agrebe™ in the dark period. Agrebe™ is a fibrous cloth that not only has the softest feel, but also seems to be the easiest material for mice to use in building their nests, compared to the other materials. Agrebe™ may present several advantages in terms of the animal welfare as well as preserving natural resources used as bedding material for experimental animals. The mice stayed on Shepherd-shack™ longer in the dark, which is a more active time for mice. The mouse is a nocturnal animal, and eating, drinking and excretion behaviour are mostly conducted in darkness [9, 17]. By staying in both cages, Agrebe™ and Shepherd-shack™, the mice suggested the differences in kinds of behavior between in the light and dark periods.

By providing animals with nesting material, the animals are enabled to use an active strategy to manipulate and to control more aspects of their environment, which is important for the effectiveness of the enrichment of their environment [14].

The most important aim of environmental enrichment is to meet the needs of essential behavior of animals in order to improve their well-being in captivity [15, 18]. Agrebe™ is easily utilized in a standard cage and thus may be a relatively simple way of contributing to the well-being of laboratory mice.

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