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Original Article

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Abstract

In the field of biomedical research, a wide variety of environmental enrichment items are available for laboratory mice to fulfill its physiological and behavioral needs which influence the outcome of animal experiments. However, to take a decision on the most suitable enrichment, researchers are likely to consider the cost of enrichment item more than animal preference and animal well-being. To develop a suitable environmental enrichment program for mice in Laboratory Animal Center, National Institute of Health (LAC-Thai NIH), we evaluated mouse preference for cages containing different types of nesting material and enrichment device. We carried out a simple preference test in groups of mice. Mice were allowed to choose three nesting materials; woodchips, paper towel and paper strip; and three enrichment devices; commercial plastic mouse house, standard plastic mouse house and cassava-based cup for 48 hours, then we evaluate where the mice positioned their nests and the amount of time they spent in the cage. The mice showed a statistical significant preference of nesting materials for paper towels than paper strip and of enrichment devices for standard plastic mouse house than commercial plastic mouse house. They also showed preference of cassava-based cup comparing with standard plastic mouse house. These data will be supporting information for environmental enrichment program of laboratory mice in LAC-Thai NIH.

Keywords: environmental enrichment, mice, preference, welfare

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บทคัดย่อ

การประเมินความพึงพอใจของหนูไมซ์ต่อชนิดวัสดุรองนอนและอุปกรณ์เพื่อการเพิ่มพูนสภาพแวดล้อม

*นวขนิษฐ์ สัจจานนท์ * วริฏฐา สงวนเรือง สมชาย สอิ้งแก้ว เรวดี บุตราภรณ์*

สำหรับงานทดสอบและวิจัยด้านวิทยาศาสตร์การแพทย์นั้น สิ่งเพิ่มพูนสภาพแวดล้อมสำหรับสัตว์ทดลองชนิดหนูไมซ์จะช่วยเติมเต็มความต้องการด้านสรีรวิทยาและพฤติกรรมซึ่งมีผลต่อข้อมูลที่ได้จากสัตว์ทดลองและส่งผลกระทบต่องานวิจัยโดยรวม การตัดสินใจเลือกชนิดของสิ่งเพิ่มพูนสภาพแวดล้อมมักถูกพิจารณาจากปัจจัยด้านราคามากกว่าปัจจัยด้านความพึงพอใจของสัตว์หรือเพื่อการเสริมสร้างสวัสดิภาพสัตว์การพัฒนาโครงการสิ่งเพิ่มพูนสภาพแวดล้อมสำหรับหนูไมซ์ของศูนย์สัตว์ทดลอง สถาบันวิจัยวิทยาศาสตร์สาธารณสุข กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุขนั้นได้ดำเนินการประเมินความพึงพอใจของหนูไมซ์ทั้งต่อวัสดุทำรังและอุปกรณ์เพิ่มพูนสภาพแวดล้อมโดยใช้วิธีการทดสอบความพึงพอใจแบบเบื้องต้นโดยให้สัตว์เลือกระหว่างกรงที่มีวัสดุทำรังจำนวน 3 ชนิด ได้แก่ ขี้กบ กระดาษและกระดาษเส้น และกรงที่มีอุปกรณ์เพิ่มพูนสภาพแวดล้อมจำนวน 3 ชนิด ได้แก่ บ้านพลาสติกเพื่อการค้า บ้านพลาสติกและถ้วยมันสำปะหลัง เป็นเวลา 48 ชั่วโมง จากการสังเกตกรงที่สัตว์เลือกทำรังรวมทั้งเวลาที่สัตว์ใช้ในกรงชนิดต่างๆ พบว่า หนูไมซ์แสดงความพึงพอใจอย่างมีนัยสำคัญทางสถิติต่อวัสดุทำรังชนิดกระดาษมากกว่ากระดาษเส้น และพึงพอใจอย่างมีนัยสำคัญทางสถิติต่ออุปกรณ์เพิ่มพูนสภาพแวดล้อมชนิดบ้านพลาสติกมากกว่าบ้านพลาสติกเพื่อการค้า นอกจากนี้ หนูไมซ์ยังแสดงความพึงพอใจต่อถ้วยมันสำปะหลังมากกว่าบ้านพลาสติก จากข้อมูลดังกล่าว ศูนย์สัตว์ทดลอง สถาบันวิจัยวิทยาศาสตร์สาธารณสุขสามารถใช้เป็นข้อมูลสนับสนุนประกอบการพิจารณาคัดเลือกวัสดุทำรังและอุปกรณ์เพิ่มพูนสภาพแวดล้อมเพื่อพัฒนาโครงการสิ่งเพิ่มพูนสภาพแวดล้อมให้เหมาะสมสำหรับหนูไมซ์เพื่องานทดสอบและวิจัยทางวิทยาศาสตร์การแพทย์ต่อไป

คำสำคัญ: สิ่งเพิ่มพูนสภาพแวดล้อม หนูไมซ์ ความพึงพอใจ สวัสดิภาพสัตว์

กลุ่มสัตว์ทดลอง สถาบันวิจัยวิทยาศาสตร์สาธารณสุข กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข จังหวัดนนทบุรี 11000
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Introduction

Environmental conditions such as housing and husbandry have a major impact on laboratory animal throughout its life and will thereby influence the outcome of animal experiments (Van de Weerd et al., 2002). Standard laboratory cages are designed to fulfill the most essential needs in a laboratory animal's life such as provision of food, water and bedding. However, animals also have physiological and behavioral needs. Physiological needs include eating, drinking, sleeping and should logically include some provision of shelter. Behavioral needs include performing behavior necessary for the maintenance of a normal physiological and psychological state (Poole, 1998), most of which cannot be met in these cages. The animals are able to perform only a part of their complete species specific behavioral repertoire (Van de Weerd et al., 1994), which can result in abnormal behavior. Furthermore, animals maintained in unresponsive environments and highly unnatural groupings are less adequate models for extrapolating experimental results to humans (Markowitz and Gavazzi, 1995). Enrichment provides a more structured environment. Enrichment can focus on several aspects of the environment such as housing animals in pairs or groups are a form of social enrichment. Foraging by scattering food particles in the bedding is a part of nutritional environment. The cage and its contents are part of the physical environment (Van de Weerd and Baumans, 1999). It is generally agreed that environmental enrichment is beneficial for the well-being of laboratory animals and that it should be applied whenever appropriate which enables them to perform more of their species-specific behavior and which gives them more control over their environment (Kaliste and Mering, 2004). The introduced enrichment should be interesting for the animals by meeting their behavioral requirements but, from the human point of view, it should be easy to provide, remove and clean (Van de Weerd and Baumans, 1995). Nesting material is an easily applicable form of enrichment for laboratory mice. Both males and females will build a nest when offered nesting materials. Nesting material is also used as a source of protection e.g. against extreme environmental temperatures, when physiological systems alone are inadequate to maintain body temperature. Nests also offer an opportunity to hide

from predators and in the laboratory to avoid aggressive con specifics, or to provide a shelter from overexposure to light. Mice in the enriched environment exhibited less anxiety-like behavior and more activity compared with mice in the standard cages (Benaroya-Milshtein et al., 2004). When applying enrichment, it is necessary to evaluate the suitability of the enrichment program, as various species or strains may respond differently to the methods of enrichment. To assess the preference of an animal for a certain feature, one can use well-designed choice tests. Specifically, this technique has been used to identify laboratory animal preferences and avoidances for specific housing conditions including cage size, bedding type, nesting type, etc. Individual animal can be offered different housing conditions in different test cages. During the test period, the movements of the animal between the test cages can be detected (Blom et al., 1992).

The aim of the present study was to investigate possible differences in preference for nesting materials and enrichment devices as a supporting data for a better development of suitable enrichment program of LAC-Thai NIH, accredited by AAALAC International. For this purpose, major strain and sex of animal used in LAC-Thai NIH were selected in a preference test for different types of nesting materials and also enrichment devices added to otherwise standard environmental conditions were evaluated.

Materials and Methods

Animals: 10 male ICR mice were used. The mice were 10-12 weeks of age. The use of animals was approved by Thai-NIH Animal Care and Use Committee (#53-013). Prior to this study, the mice were housed in groups of five animals in shoebox cages (9x14x5.5 inch) on corncob contact bedding that was changed twice a week. Cages were supplied with food pellets and RO water in bottle *ad libitum*. The animals were kept in conventional rooms with controlled photo period (12: 12 L: D, lights on at 06:00 hour, approx. 200 lux at 1 m above the floor), temperature (20-22^{0}C), relative humidity (50-60%) and ventilation (12-15 air changes per hour). Environmental conditions in the experimental rooms were similar. One day prior to a preference testing, each mouse was introduced into the test system for 3 hour in order to familiarize the mice with test system and they have a recess time, not less than 7 days, between each experiment.

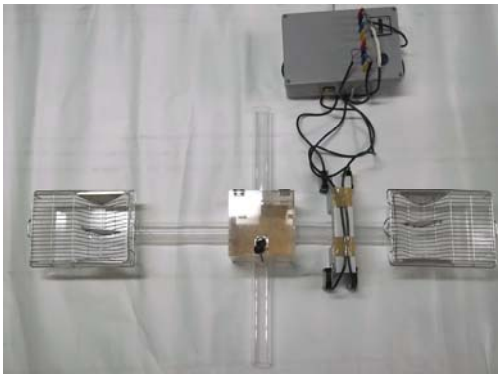

Figure 1 The preference test system (top view).

Preference test system: Three nesting materials and three enrichment devices were evaluated with the use of two-cage choice housing system (Fig 1). It consisted of two test cages connected by transparent tubes (inner dimension 4.5 cm, 20 cm long) to a central cage (15x15x16 cm). The test system was gently rotated 180 degrees every 24 hours to prevent bias due to external influences in the experimental room which could interfere with the choice behavior of the mouse. The test cages were supplied with 150 g of corncob bedding, a food hopper with 50 g of food pellets and 250 g RO water in bottles. The central choice cage had no food, water or bedding. The movements of the

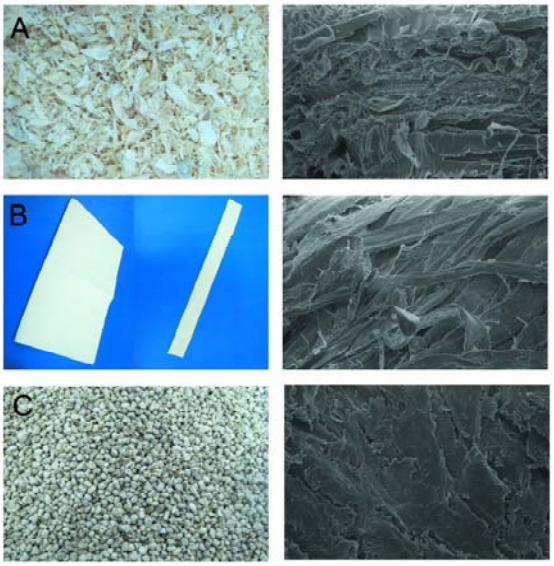

Figure 2 The three nesting materials tested and background bedding material. A: Woodchips (left), scanning electron micrograph x 500 (right), B: Paper series; Paper towel and Paper strip (left), scanning electron micrograph x 500 (right), C: Corn cob (left), scanning electron micrograph x 500 (right).

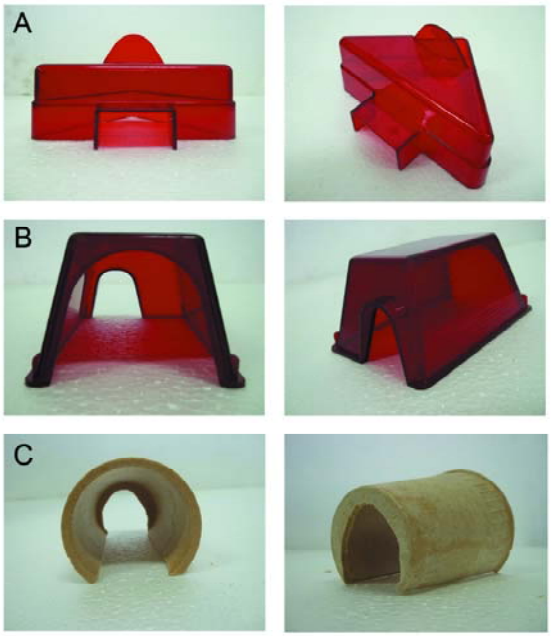

Figure 3 The three enrichment devices tested. A: Commercial plastic mouse house, B: Standard plastic mouse house, C: Cassava-based cup.

mice between the test cages were detected automatically by means of photo-electrical devices in the passage tubes.

Preference test; nesting materials and enrichment devices: We used one system containing positions for two cages with a photo-electrical device for measuring dwelling time for one cage. Mouse was introduced into the test system by placing into the central choice cage between 10:00 and 12:00 and tested individually during 48 hours. To discourage the mice from remaining in the central choice cage, we did not place bedding, food or water. The behavior of one animal was observed via video camera for the first 2 hours, so that movements of the mouse could be followed in the test system. We also observed and monitored the mouse and test system 3 times daily. Food and water of each test cage were weighed before and after the experiment.

Two assessments were performed to test types of each nesting materials and enrichment devices. For the nesting material assessment; three nesting materials (woodchips and paper series; paper towel and paper strip) were tested by comparing woodchips with paper towel (experiment 1) and paper towel with paper strip (experiment 2). For the enrichment device assessment; three enrichment devices (cassava-based cup and plastic mouse house series; standard and commercial plastic mouse house) were tested by comparing standard with commercial plastic mouse house (experiment 3) and standard plastic mouse house with cassava-based cup (experiment 4). Table 1 describes the materials and gives the amounts provided per cage and Figs 2 and 3 show nesting materials and enrichment devices.

Scanning Electron Microscopy (SEM): The ultrastructure of bedding and nesting materials were studied using SEM. Specimens (corn cob, woodchips and paper) were attached to aluminum stubs with double-stick tape and coated with gold (Au) in a sputter-coating apparatus before being viewed with a JEOL JSM-5910LV scanning electron microscope (JEOL: Japan).

Statistical analysis: Dwelling time per cage data was collected from individual mouse at 24 and 48 hours. The dwelling time data were analyzed which of the cages were preferred or avoided using paired t-test. Statistical significance was preset at $p < 0.05$. Data on food and water intake were not transformed because they were normally distributed.

Results

For the nesting material assessment (Fig 4) at 24 hours, there were no significant differences in cage preferences (dwelling time) between paper towel and woodchips of experiment 1, paper towel and paper strip of experiment 2, (paired *t*-test, $p < 0.05$). At 48 hours, comparing between woodchips and paper towel in experiment 1, no significant difference in dwelling times was found but we inferred that mice preferred paper towels because 60% of the mice dragged paper towels from one cage to the other cage containing woodchips to combine two types of nesting material. Comparing between paper series (paper towel and paper strip) in experiment 2, the cage containing paper towel was found to significantly affect mouse preference with mice spending greater time than in the cage containing paper strip, $p < 0.05$. Anyway, all mice showed the same activity shredding sheet of paper towel and also paper strip to build nest. Also, mice showed nest building behavior with all nesting materials. No significant difference of all nesting materials from 24 to 48 hours.

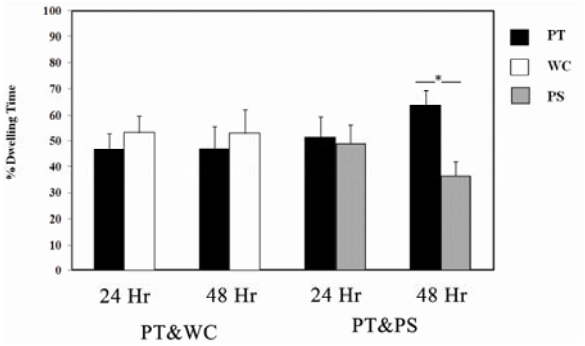

Figure 4 The percentages of dwelling time of nesting material assessment
PT: Paper towel, WC: Woodchips, PS: Paper strip; -*-$p < 0.05$ at 48 hours of paper towel compared with paper strip (paired *t*-test, values presented mean±SEM).

Table 1 Nesting materials and enrichment devices

Type	Material	Trade name	Appearance (size)	Amount
Nesting materials	Woodchips	Versele-Laga™, Belgium	Wood (variable)	50 g
	Paper towel	River pros™, Thailand	Sheet (20 x 24.5 cm)	1 piece
	Paper strip	River pros™, Thailand	Strip (2 x 24.5 cm)	10 piece
Enrichment devices	Commercial plastic mouse house	Techniplast™, Italy	Transparent, polycarbonate, triangular-shaped box	1 piece
	Standard plastic mouse house	Bio-Serv™, USA	Transparent, polycarbonate house	1 piece
	Cassava-based cup	KU-GREEN™, Thailand	Opaque, biodegradable cup (83 mm D x 52 mm H)	1 piece

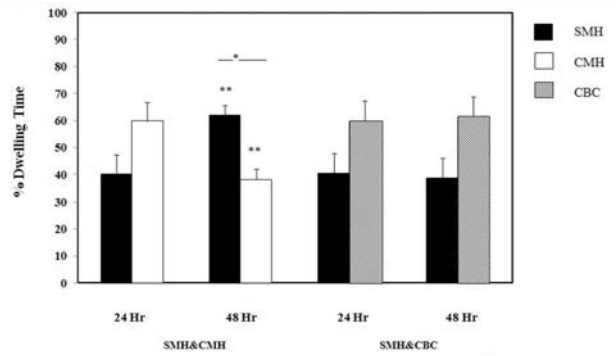

Figure 5 Percentages of dwelling time of enrichment device assessment
SMH: Standard plastic mouse house, CMH: Commercial plastic mouse house, CBC: Cassava-based cup
-*- $p < 0.05$ at 48 hours of standard plastic mouse house compared with commercial plastic mouse house (paired *t*-test)
** $p < 0.05$ from 24 to 48 hours of standard plastic mouse house and commercial plastic mouse house (paired *t*-test) (values presented mean±SEM)

For the enrichment device assessment (Fig 5) at 24 hours, there were no significant differences between standard and commercial plastic mouse house of experiment 3, cassava-based cup and standard plastic mouse house of experiment 4. At 48 hours, comparing between plastic mouse house series in experiment 3, it showed significant differences between commercial and standard plastic mouse house. The mice preferred standard than commercial plastic mouse house. Comparing between cassava-based cup and standard plastic mouse house in experiment 4, the result showed no statistical significant difference. Although the mean of data showed as it seemed to make a difference but the range of standard deviation is so wide that they showed no significant difference using paired t-test analysis. The standard plastic mouse house and cassava-based cup were clearly preferred by mice than commercial plastic mouse house. There were significant differences from 24 to 48 hours standard and commercial plastic mouse house in experiment 3. The mice showed more activities such as sleeping in and turning over with cassava-based cup than plastic mouse house series.

In every experiment, the behavioral data on video tape of the first 2 hours of experiment showed an exploration of mice cages. Observing the mouse and test system 3 times daily, all mice showed nest building behavior within the first 24 hours using nesting materials and clearly decided to choose one cage within 48 hours. The mice used the preferred cage to sleep in and ate mostly in the cage they did not live in. Eating and drinking behavior of the mice was in normal range. Water intake did not differ between the cages.

Discussion

The results of this study indicate that mice preferred cages with paper towel as nesting material than paper strip. The mice did not make a significant choice between the two nesting materials offered; paper towel and woodchips, but in most cases combined them. We exclude the data that mice dragged paper towel in the experiment because the mice showed their decision to combine both nesting materials. All mice dragging paper towel to cage containing woodchips because the structure of paper towel might be easier to move than woodchip. Behavioral observation showed that manipulation of the bedding and resting in nests was performed mostly on paper towel. The fact that some animals combined nesting materials might suggest that mice preferred to make more complicated nest or there is not a clear preference for the nature of nesting materials e.g. paper or wood but that other features of the nesting materials such as the structure e.g. sheet or strip also play a role. The results suggest that the nature of the nesting materials might be less important than the structure. On the other hand, the structure may be important because it determines the nesting ability of the material. In the present study, the characteristic feature which the preferred nesting material has in common is that the mice can manipulate them to build nests, and by doing this, they are able to construct their own environment. Another aspect of the nesting materials which could be an important criterion for selection by the mice is the degree of light absorption. Mice are nocturnal animals which often prefer to hide and sleep in dark places during daytime. However, the nesting materials in the present study allowed some penetration of light but the paper towel could provide a shelter for light, completely covered by the materials of their nests. Not only shelter for light, paper towel as nest can also control temperature of microenvironment for mice. Besides, a recessive animal can use shelter as a safe area for hiding that may increase its sense of security. When aggressive males are being used, individual housing is common practice. Single housing in order to avoid aggression between male mice is a solution with evident negative consequences for the animals, the presence of nesting material could partly compensate for the deprivation of social contact (Van Loo et al., 2004). Some study indicated that the male ICR mice showed preference in reusable cloth as nesting material than recycled-paper (Kawakami et al., 2007). In Blom's study, the mice showed a preference for shredded filter paper in comparison with smaller particle bedding material (Blom et al., 1996). The study by Blom (1992) showed that 65% of the time is spent on sleeping, grooming and digging behavior. In the present study, nest building behavior could be performed with the nesting materials and digging was less frequently observed. It is possible that female mice would demonstrate a different preference, however, results from previous preference tests (Van de Weerd et al., 1997) indicated that enrichment preferences of male mice did not differ drastically from those of female mice. This is able to use an active strategy to manipulate and control more aspects of environment for mice and be a simple method to contribute to the well-being of laboratory mice.

In view of enrichment device results presented here, it presumes that the cassava-based cup and standard plastic mouse house have features which provide added attractive value to mice. The commercial plastic mouse house seems to lack these features. It is interesting that the mice showed significantly lower preference for commercial plastic mouse house comparing with standard type which has the same material. This may suggest that the shape of enrichment device is an important factor for animal needs. Anyway, the material of enrichment device is also important. Some study indicated a strong preference by mice for paper-based triangular-shaped enrichment device more than commercial plastic mouse house which has almost the same shape (Van Loo et al., 2005). Commercial plastic mouse house has more complex structure, triangular-shaped box with a small tunnel opening on the long side with an extra triangular opening in the top, than standard type. The top opening allowed light penetrating to the cage floor. Tunnel opening is only 3 cm in height which made it difficult for adult male mice to pass so they only used top opening to enter the box. These factors might affect the mice preference. Although the cassava-based cup and standard plastic house did not significantly show which were more preferred by the mice in statistical analysis but the mean of data clearly showed that the mice preferred cassava-based cup. Cassava-based cup and standard plastic mouse house differ with regard in not only construction material; rough, opaque and eatable vs smooth, transparent and uneatable but also weight; light vs heavy, respectively. These features make it possible to manipulate the cassava-based cup while the plastic mouse house is more rigid. The plastic mouse house always stayed in position once placed in the cage while the mice usually moved around and manipulated the cassava-based cup. We also observed that mice turned over the cassava-based cup to create their own rocking chair. These observations provide evidence that enrichment device increases environmental control for mice which is an important stress-reducing propensity (Sambrook and Buchanan-Smith, 1997).

It is upon individuals who use and care for laboratory animals to seek to improve the quality of the captive environment. To that end, well-designed and carefully communicated enrichment approaches are required. More data are needed to provide information related to the effects of specific enrichment programs on the animal, on specific animal species and on experimental results. The most important aim of environmental enrichment is to make the animals feel secure and give them a sense of control within a complex, challenging environment that meets essential species-typically behavioral needs of animals in order to improve their well-being in captivity. The selection for suitable enrichment items of laboratory animal center should be adapted from the originals, for example cassava-based cup was adapted from standard plastic mouse house and paper towel was adapted from commercial paper nesting material. The reason for enrichment item adaptation is harmonization between animal needs with scientific evidence and human needs, economy and ergonomics. The advantage of cassava-based cup and paper towel is that they can produce in Thailand, therefore, they are inexpensive. They are also disposable, making them easier to be managed than other items. Anyway, both of them need to have analysis of contaminations; aromatic hydrocarbon, heavy metals, bleach, etc.; to make sure that it is safe for animals when using in long-term experiment. To comply with the animals' needs while keeping in mind human requirements, we propose that the ultimate enrichment item should be tried on the basis of knowledge before use in laboratory animal center.

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References

Benaroya-Milshtein N, Hollander N, Apter A, Kukulansky T, Raz N, Wilf A, Yaniv I and Pick C 2004. Environmental enrichment in mice decreases anxiety, attenuated stress responses and enhances natural killer cell activity. Eur J Neurosci. 20: 1341-1347.

Blom H, Van Tintelen G, Van Vorstenbosch CJ,Baumans V and Beynen AC 1996. Preferences of mice and rats for types of bedding material. Lab Anim. 30(3): 234-244.

Blom H, Van Vorstenbosch C, Baumans V, Hoogervorst M, Beynen A and Van Zutphen LF 1992. Description and validation of a preference test system to evaluate housing conditions for laboratory mice. Appl Anim Behav Sci. 35: 67-82.

Kaliste E and Mering S 2004. The welfare of laboratory rats. In: The Welfare of Laboratory Rats. Kluwer Academic Press. Dordrecht: 153-180.

Kawakami K, Shimosaki S, Tongu M, Kobayashi Y, Nabika T, Nomura M and Yamada T 2007. Evaluation of bedding and nesting materials for laboratory mice by preference tests. Exp Anim. 56(5): 363-368.

Markowitz H and Gavazzi A 1995. Eleven principles for improving the quality of captive animal life. Lab Anim. 24(4): 30-33.

Poole T 1998. Meeting a mammal's psychological needs: Basic principles. In: Meeting a Mammal's Psychological Needs: Basic Principles. Smithsonian Institution Press. Washington. 94 pp.

Sambrook T and Buchanan-Smith H 1997. Control and complexity in novel object enrichment. Anim Welf. 6: 207-216.

Van de Weerd H and Baumans V 1995. Environmental enrichment in rodents. In: Environmental Enrichment in Rodents, 2. AWIC Resource Series: 145-149.

Van de Weerd H and Baumans V 1999. Evaluation of environmental enrichment for laboratory mice. AWIC Bulletin. 9(3-4): 18-19.

Van de Weerd H, Aarsen EL, Mulder A, Kruitwagen CL, Hendriksen CF and Baumans V 2002. Effects of environmental enrichment for mice: Variation in experimental results. J Appl Anim Welf Sci. 5(2): 87-109.

Van de Weerd H, Baumans V, Koolhaas JM and Van Zutphen LF 1994. Strain specific behavioural response to environmental enrichment in the mouse. J Exp Anim Sci. 36(4-5): 117-127.

Van de Weerd H, Van Loo PL, Van Zutphen LF, Koolhaas JM and Baumans V 1997. Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. Physiol Behav. 62(5): 1019-1028.

Van Loo PL, Blom H, Meijer M and Bauman V 2005. Assessment of the use of two commercially available environmental enrichments by laboratory mice by preference testing. Lab Anim. 39 (1): 58-67.

Van Loo PL, Van de Weerd H, Van Zutphen LF and Baumans V 2004. Preference for social contact versus environmental enrichment in male laboratory mice. Lab Anim. 38 (2): 178-188.