The use of aspen blocks and tubes to enrich the cage environment of laboratory rats

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Abstract

Female and male outbred Wistar rats (n=48) were allocated into three groups at weaning; control, tube and block groups (n = 8 males and 8 females in each). Animals were conventionally housed for five weeks in groups of four per cage with either an aspen tube (20x12x12 cm) or an aspen block (6x6x6 cm) in addition to the aspen bedding. The control animals had no enrichment items in their cages. The use of tube and block was assessed by measuring both the volume gnawed and via video recordings, which were done by the instantaneous sampling method at 1 min intervals. The growth of the animals was followed by weighing the animals three times during the study. Rats with the tubes in their cage spent over 80 % of their time during light period inside the tube and over 20 % during dark period. Furthermore, when the lights were turned off, rats increased other contacts (on and beside) with the tube from 5 % to about 40 %. Animals with blocks spent about 3 % of their time during the light period on top of the block or in its vicinity and about 11 % during the dark period. The amount gnawed was essentially the same with both items and increased slightly with time. The enrichment items did not have any effects on the growth of the animals. In conclusion, the enrichment items have the advantage of not introducing any extra or new compounds into the cage environment, since they are made from the same raw material as the bedding. The rats used the items not only for gnawing but also for other activities. The inside of the tube was mainly used as shelter from the light. The aspen tube seemed to have more enrichment value for rats than the aspen block, since it allowed a wider range of behaviour patterns to be expressed. These items were reusable, economical and species-appropriate for enriching the cage environment of laboratory rats.

Keywords: environmental enrichment, gnawing, rat.

Introduction

During two previous decenniums a fundamental concept was introduced into laboratory animal care. One should always strive to limit the materials to which the animals would be exposed to an absolute minimum and to favour inert materials. The rationale was to apply two concepts of the 3Rs; refinement and reduction (*Russell & Burch*, 1959) in order to decrease the number of animals needed, but still achieve reliable results. This decade has seen concern about how one can enrich the living conditions of laboratory animals. One would have expected that these recent developments would have taken into consideration the earlier concept in the design of enrichment objects. This has not been the case.

There seems to be a general consensus that standard housing conditions for laboratory animals are barren and lack psychological or physiological stimulation. This conclusion is stated in a multitude of reference guidelines and recommendations on housing conditions for several species (*European Convention*, 1986; *Hubrecht*, 1993; *BVAAWF/FRAME/RSPCA/UFAW Joint Working Group*, 1993; *Home Office*, 1995). The European Commission's international workshop recommends that rodent cage environment should satisfy the physiological and ethological needs of resting, grooming, exploring, hiding, searching for food and gnawing (*Brain et al.*, 1993).

However, while attempting to enrich the environment, often the only real guideline followed has been the acceptability and use of the enrichment items by the animals themselves and the imagination of the researcher. Almost anything has been suggested - and apparently used; cans, toilet paper rolls, PVC- or plastic-tubes, glass jars, cardboard and polypropylene boxes (*Scharmann*, 1991; *Brooks et al.*, 1993; *Chmiel & Noonan*, 1996; *Townsend*, 1997). Consequently, the source of the materials is - to say the least - variable. Yet, the potential confounding effects of these materials on experimental results have received little attention (*Townsend*, 1997; *Manser et al.*, 1998a).
One rather obvious solution to this dilemma is to use a material already present in the cage, *i.e.* bedding. It is surprising that so few of the studies on wooden enrichment devices have acknowledged this, even though they may have used a different type of wood (*Orok-Edem & Key*, 1994; *Chmiel & Noonan*, 1996). It is well-documented that softwoods (*e.g.* pine and red cedar) can contain more enzyme inducing or cytotoxic compounds than hardwoods (*e.g.* alder and aspen) and thus can affect drug metabolism (*Ferguson*, 1966; *Vesell*, 1967; *Cunliffe-Beamer et al.*, 1981; *Törrönen et al.*, 1989; *Potgieter et al.*, 1995). Hence aspen, when used also as bedding, appears to be a more suitable starting point for making enrichment items.
In addition to the material used for their construction, the function, structure and nature of enrichment items are crucial. Rats seek out and spend more time with chewable objects *e.g.* blocks with holes (*Chmiel & Noonan*, 1996). Furthermore, nest-boxes are more attractive than other parts of the cage, which might be due to possibility to escape from other animals and light (*Manser et al.*, 1998a), provision of elements to satisfy wall-hugging tendency or option to move in a third-dimension (*Townsend*, 1997). This study was designed to assess the applicability and use of two common enrichment objects – a tube and a block - made of the same material as the bedding.

Materials and Methods

Animals and environment

A total of 48 barrier bred, but conventionally housed outbred Wistar (WH, Hannover origin) rats (National Laboratory Animal Center, Kuopio, Finland) were used. The animals were chosen from eight litters, three females and three males from each and allocated into three groups at weaning; control, tube and block group (8 males and 8 females in each). The rats were housed in stainless steel solid bottom cages (48x28x20 cm with a wire lid) in groups of four (n = 4 cages per group).
The rats were three weeks old at the beginning and eight weeks at the end of the study. The bedding used (1,2 l per cage) was aspen chips (4HP, Tapvei Oy, Kaavi, Finland), changed twice a week on Mondays and Fridays. The animals were housed at an ambient temperature of 20±2 °C and relative humidity of 42-72 %. The light/dark cycle of the animal room was 12:12 hours with lights on at 7.00. Commercial diet (R36, Lactamin AB, Södertälje, Sweden) and tap water in polycarbonate bottles were available *ad libitum*.

Enrichment items

In addition to the bedding material, two different enrichment items were used - a block (6x6x6 cm with penetrating drilled holes, diameter of 1.9 cm on each side) and a rectangular tube (20x12x12 cm with the 1.5 cm wall thickness) made of dried aspen board (Fig 1). The walls of the tube were pinned together with aspen pins in predrilled holes *i.e.* no glue was used. The size of the tube was chosen so that even large rats (about 250 g) could enter inside the tube but still there would be enough space to move. The size of the block was based on an earlier study (*Kaliste-Korhonen et al.*, 1995), and chosen so that it would last for at least one week. The shape was modified from the study by *Chmiel & Noonan* (1996). The enrichment items were placed into the cages on the second day of the study. The items were replaced once every week on Wednesdays.

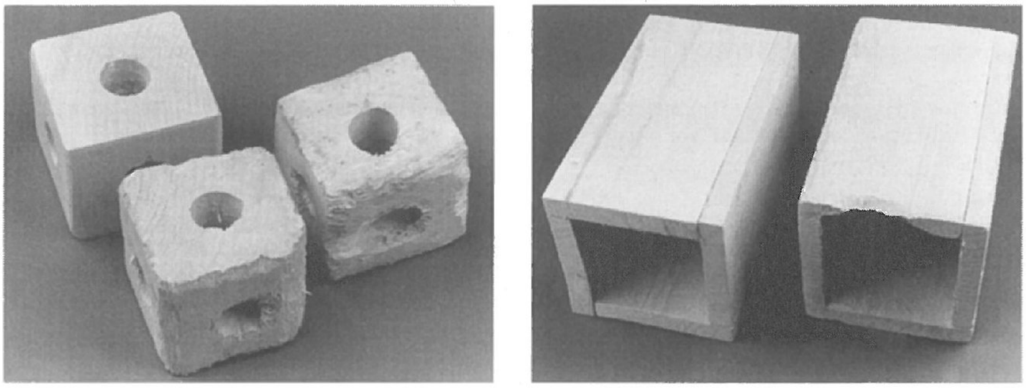

Fig 1. The enrichment items - aspen blocks (6x6x6 cm) and rectangular tubes (20x12x12 cm) made of dried aspen board. One of each items is unused.

Use of items

The behaviour of the animals in two block cages (one female and one male cage) and in two tube cages (one female and one male cage) was video recorded (S-VHS LC295SN video camera, Grundig, Germany) once a week on four weeks with a time-lapse recording system at 1s/1 min intervals. The recordings took place from 16.00 until 01.30, *i.e.* three hours were during the light period and six and a half hours during the dark period. During the recording, the cages were placed on the floor of the animal room with three red 25 W lamps used during the dark period to permit viewing. The temperature was measured at the level of cages to ensure that the lights did not warm the cages.

The data was analysed with instantaneous sampling at 1 min intervals and the number of animals inside, on or beside (the paws or head of the animals were in contact with the item) the tube or not in contact with the tube (*i.e.* elsewhere) were counted. The same behaviour variables were measured from the block group, except for the "inside" -variable, since it was not possible to go inside the block. The first half an hour of the recording was omitted from the analysis, since the presence of researcher and the recording arrangements clearly affected the behaviour of the rats.

Gnawing of the objects was assessed weekly by measuring the volume gnawed. This was done by pasting filling material (Oiva Pikasilote Sadolin, Nordsjö AB, Sweden) on the places gnawed. After drying, the filling material was scraped off and the volume was measured.

Light intensity

The light intensities inside the cages during the light period varied from about 100 lux (top row in the cage rack, beside the items) and 30 lux (top row, under the food hopper) to 20 lux (middle row, beside the items). Inside the tubes, the light intensity was below 5 lux. During the dark periods the light intensities were always below 1 lux. The light conditions during the video recordings were equivalent to the top row conditions.

Growth

Animals were weighed three times during the study - at the age of three, seven and eight weeks.

Statistical analysis

The data were processed with SPSS for Windows statistical package (Release 6.1.4, SPSS Inc., Chicago, IL, USA). The normality of the data was tested with Kolmogorov - Smirnov test. The statistical analyses used were repeated measures analysis of variance with univariate test (RMU), multivariate analysis of variance with univariate test (MANOVAU), multivariate analysis of variance with multivariate test (MANOVAM), paired t-test (PT) and Friedman-test (F). These abbreviations are used in the results.

Results

Daily behaviour

Figure 2 presents the relative times (%) that the animals spent in different parts of the cage in relation to the items during light (16.00-18.59 hours) and dark periods (19.00-1.30 hours). The results are expressed separately for females with tubes (Fig 2a and b, n=4), males with tubes (Fig 2c and d, n=4) and combined results of both sexes for animals with blocks (Fig 2e and f, n=8).

Light period - Animals with tubes spent about 87 % of their time inside the items - this behaviour pattern was not possible in cages with blocks. Furthermore, the animals which had the tubes spent less time elsewhere in the cage (mean 9 %, i.e. males and females combined) than animals with blocks (97 %) (p=0.000, MANOVAU). The times spent on top or beside the devices were similar in both enrichment groups (p=0.541 and p=0.156, MANOVAU, respectively). No gender differences were detected during the light period.

Dark period - In cages with tubes, male rats spent more time in other parts of the cage (elsewhere) or beside the tube (total 54 %) than females (total 32 %), but still less time than animals with blocks (total 93 %) (p=0.005, MANOVAM). Correspondingly, females spent more time on top of the tube (45 %) than males (22 %), though this was longer than the time that animals spent on top of the block (7 %) (p=0.000, MANOVAU). Both females and males in cages with tubes spent equal amounts of time inside the tube (23 % vs. 24 %, respectively) (p=0.765, MANOVAU).

Light vs. dark period - Both females and males with tubes spent more time inside the tube during the light period (mean 87 %) than during the dark period (mean 24 %) (p=0.000, PT). There were, however, some gender differences in the times spent elsewhere, on or beside the tubes during the dark periods (see above). In general, the animals with tubes spent more time beside or on top of the tube during the dark period (mean 40 %) than during the light period (mean 4 %) (p<0.03 for all the other variables except for females beside the tube, PT). Animals with blocks spent more time on and beside the block during the dark period (total 11 %) than during the light period (total 3 %) and less time elsewhere in the cage (89 % vs. 97 %, respectively) (p<0.001 for all variables, PT).

Behaviour by time - The behaviour of the animals during different times of the day is presented in figure 3. Both females and males decreased the time spent inside the tube immediately after the lights went off at 19.00 hours and increased their presence on top of the tube, in its vicinity or elsewhere in the cage (p=0.001, F, Fig 3a and b). The animals with blocks were also detected more frequently on or beside the block after the dark period had started (p=0.001, F, Fig 3c).

Gnawing

The rats used the aspen tubes and blocks also for gnawing. The amount gnawed (Fig 4) was essentially the same with both objects (p=0.902, RMU) and increased slightly with time (p=0.006, RMU). Both females and males gnawed the objects similarly (p=0.828, RMU).

Growth

The enrichment items did not have any effects on growth of either sex when compared to control animals (p=0.668, RMU, Fig 5). The male rats were heavier than the females after the age of seven weeks till the end of the study (p=0.000, RMU).

Discussion

It has been suggested that environmental enrichment can lead to a lack of standardisation and increase the variables present in an experiment (*Batchelor*, 1997; *Hubrecht*, 1997). Nevertheless, the popularity and implementation of enrichment programmes have increased tremendously during the last decade. In this process, one obvious solution to introduction of unnecessary variables has been ignored; *i.e.* enrichment with existing materials. This study focuses on applicability of

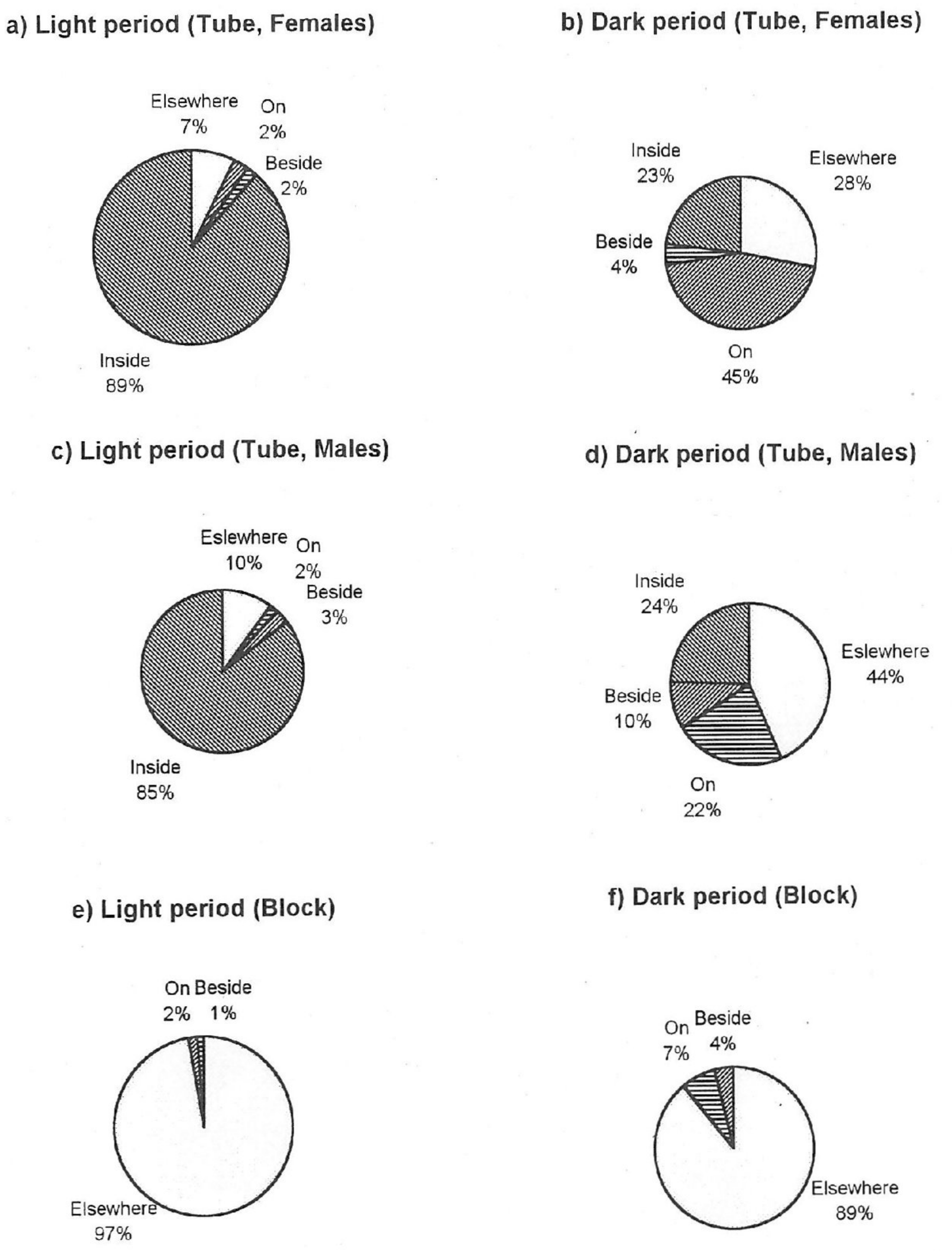

Fig 2. The relative times (%) animals spent in different parts of the cage during light (16.00-18.59 hours) and dark periods (19.00-1.30 hours) for females with tubes (a and b, n=4), males with tubes (c and d, n=4) and combined results of both sexes for animals with blocks (e and f, n = 8).

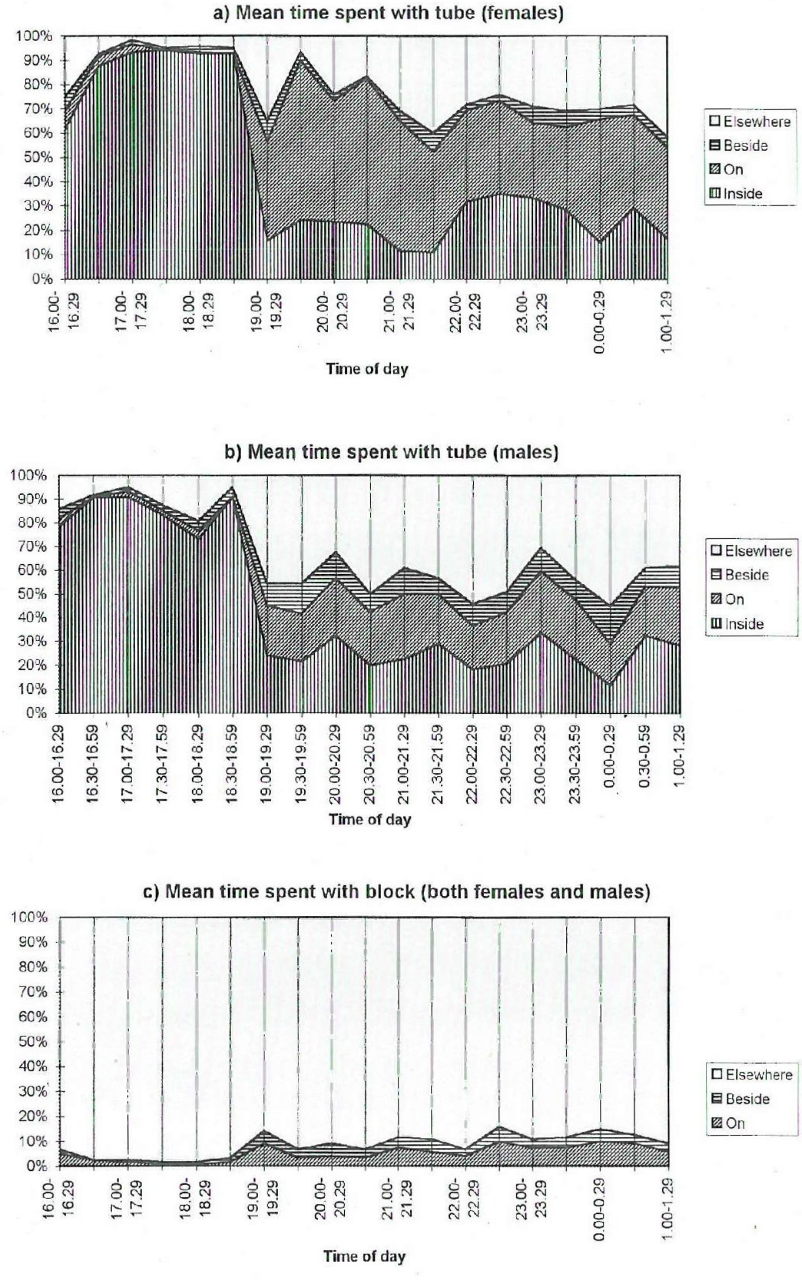

Fig 3. The behaviour by time for females with tubes (a, n=4), males with tubes (b, n=4) and combined results of both sexes for animals with blocks (c, n=8). The lights went off at 19.00.

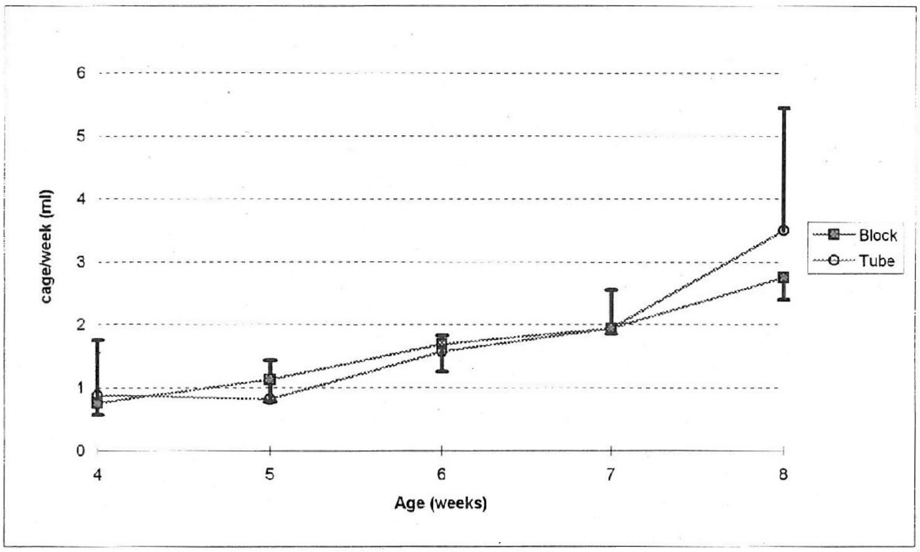

Fig 4. The mean (± SD) amount gnawed per week (ml/cage) for cages with tubes (n=4) and blocks (n=4).

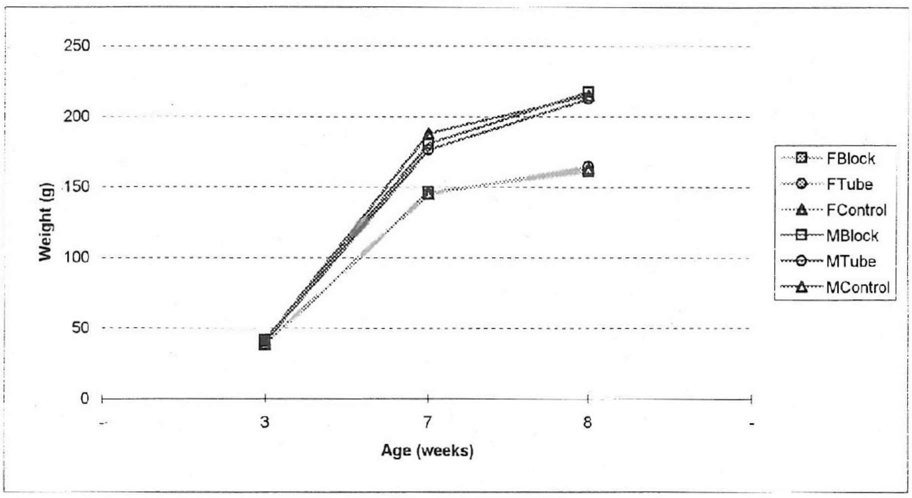

Fig 5. The mean body weights (g) at the age of three, seven and eight weeks for females (F) and males (M) with blocks, tubes and controls (n=8 animals per group).

two enrichment items made of aspen - the same raw material the rats have as bedding.

Aspen was a natural choice, since it is commonly used as bedding. As a hardwood it is a more suitable material for enrichment than softwoods. The enzyme-inducing effects of softwood beddings were first described decades ago (*Vesell*, 1967) and subsequently confirmed in several studies (*Cunliffe-Beamer et al.*, 1981; *Weichbrod et al.*, 1988). Some volatile organic compounds have been reported to be the causative agents (*Bang & Ourisson*, 1975). If one has to use softwood, the best way to reduce the volatile organic compounds is by autoclaving (*Nevalainen & Vartiainen*, 1996), but even then the concentrations are much higher than with hardwood. Both tubes and blocks were autoclaved mainly for hygienic reasons and they kept their structure even after ten autoclaving cycles.

The tube form was devised since rats have strong preference for cages containing a shelter (*Townsend*, 1997). These shelters are preferred to other parts of the cages in order to escape other animals in the cage or bright light (*Manser et al.*, 1998a). The tube size was large enough to allow the entry of all rats without hindering their access to other parts of the cage. The shape of the block made it easy to manufacture, animals could gnaw it and they could get a good grip of it in order to move it around. Furthermore, rats could stand on top of the block. Both items were large enough and thus it was unnecessary to replace them at times other than normal cage changes.

The rats used the tubes as a shelter especially during the light periods. Rats are nocturnal animals and the light intensity inside the tube was many times lower than that in other parts of the cage. The light intensity under food hopper was also about half of that present elsewhere in the cage and rats without shelters are known to spend a considerable proportion of their time during the light period under a food hopper. Furthermore, all four animals in the cage tried to squeeze themselves inside the tube at the same time. Therefore, the behaviour of animals does not support the hypothesis that the rats were escaping their cage mates but it seems that they were rather escaping the light. On the other hand, the shelter provides a hiding place from people. This, however, raises difficulties in the routine inspection of the animals - a problem which is partly resolved in opaque perspex nest-boxes (*Manser et al.*, 1998b).

The animals' control over its microclimate is suggested to have an impact on welfare (*Brain et al.*, 1993; *Townsend*, 1997; *Manser et al.*, 1998a). The possibility to go inside the tube enabled the animals to control their microclimate at least with respect to the light intensity to which they were exposed. Since the temperature or humidity inside the tube were not measured, it is not possible to say if these factors affected the attractiveness of the tube.

The rats spent more time with the aspen tube than they did with the aspen block. This may be due to the larger variety of behaviours possible in cages with a tube. The animals could go inside the tube - a behaviour, which was not possible with block. Furthermore, the larger size of the tube enabled the animals to climb on top of the tube more easily. However, due to the larger size of the tube, it may also be more difficult to avoid being in contact with it, especially when the animals grow. Since the rats in this study spent also a considerable proportion of their time elsewhere in the cage, it appears that the animals use the aspen tubes more than the aspen blocks.

Enrichment design should also engage animals in beneficial ways such as evoking exploratory behaviour (*Chmiel & Noonan*, 1996). Both enrichment items in this study encouraged the rats to explore the environment above the cage floor and also gave them the opportunity to move in a third-dimension. The rats climbed on top of the items and had other contacts with them, showing that the items encouraged explorative behaviour. The increased exploratory behaviour may be due to decreased fearfulness (*Townsend*, 1997) and the ability to perform a variety of behaviour patterns may enhance sensorimotor skills (*Prior & Sachser*, 1994/95).

Enrichment objects are expected to be species appropriate (*Line*, 1987) and encourage natural behaviours (*Chmiel & Noonan*, 1996). In this study, both enrichment items were used for gnawing, which is a natural behaviour of rodents. The gnawing increased slightly during time, which is logical when animals grow. The amount gnawed, however, was quite limited, which is probably due to the presence of bedding material. *Kaliste-Korhonen et al.* (1995) have shown that wooden blocks were not extensively gnawed in cages with contact bedding, but gnawing increased about threefold on a grid floor without contact bedding.

When enrichment items are chewable, their chemical composition is an important factor, which should be taken into consideration. Objects can also contain other possibly harmful substances in addition to volatile compounds. For example, toilet paper rolls are made of recycled paper, which frequently contains residues from the processing. The glue used in these rolls contains sodium aluminosilicate and some silicate-type sorbents have been shown to reduce the toxicity of aflatoxin (*Kubena et al.*, 1993). Furthermore, if the rolls are collected from the toilets and given to the animals without autoclaving, an obvious hazard is microbial contamination. The items in this study did not contain any extra chemicals or materials - the block was made of solid aspen board and the tube was assembled from four boards attached together with aspen pins, *i.e.* no glue was used. Furthermore, these items did not have any effect on the growth of the animals, which might be the case if toxic effects had been present.

Finally, economical aspects have to be considered when a large enrichment programme is to be implemented. The enrichment items in this study were durable and easy to clean; rinsing with water followed by autoclaving would remove most olfactory substances and other animal based residues. These items were usable and kept their structure even after ten autoclaving cycles. Thus, the enrichment shapes can be used for several weeks at a reasonable cost.

In conclusion, the aspen enrichment items have the advantage of not introducing any extra or new compounds into the cage environment, since they were made from the same material as the bedding. The wooden shapes were used for gnawing and animals had also many other contacts with them. The inside of the tube was used as a shelter against light, but rats used it also for hiding during dark periods. Furthermore, the tube allowed a wider range of behaviour patters and seemed to have more enrichment value than the block. Consequently, these objects seem to fulfil the requirement of safe and species-appropriate environmental enrichment item (*Chmiel & Noonan*, 1996).

Acknowledgements

The authors wish to thank Mrs. Tuija Suikka for her assistance during the video tape analysis.

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