The Ability of SD-Rats to Distinguish Between Three Different Housing Environments

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Summary

Since 1986, when the Council of Europe gave the first provisions for housing of laboratory animals, the focus on housing conditions has increased with emphasis on the size of primary enclosures such as cages or pens as well as the complexity of the enclosure. Today European legislation dictates the minimum amount of enrichment to be present in cages for different species.

The aim of this study was to evaluate the influence of different enrichment schemes on growth rate, water consumption, muscle strength and preference in rats, after items such as hides, nesting material, increased cage height and shelves had been introduced to the cage environment.

The study demonstrated that rats spend more time in the extra-enriched cages compared to the non-enriched cages, whereas no differences in the dwelling time between the two types of enriched cages could be detected. When present in the cage, the built-in shelf was used extensively (over 40% of the observations) although no specific preference for the extra-enriched cage was detected.

No differences in weight gain and water consumption could be detected between rats in the three different housing conditions, although there was a slight increase in muscle strength for the standard-enriched housed rats.

Introduction

Since 1986, when the Council of Europe gave the first provisions for housing of laboratory animals, the focus on housing conditions has increased with emphasis on the size of primary enclosures such as cages or pens as well as the complexity of the enclosure. From an animal welfare point of view increased complexity in the structure rather than just additional space is preferable (*Baumans, 2005*; *Bergmann et al., 1995*) to allow a wider species-specific behavioral repertoire (*Hutchinson et al., 2005*; *Mench, 1994*). Animals housed in impoverished environments without the opportunities to perform a species-specific behavior may experience increased stress and impaired welfare (*Moncek et al., 2004*; *Würbel, 2001*). Laboratory animals' need for enriched housing conditions which give opportunities for more species-specific behavior has therefore been implemented in Appendix A of the Council of Europe (CoE) convention (*Council of Europe, 1986*), which forms the basis for European legislation. The resolution from 1997 demands that *'special relevance should be given to the enrichment of the environment of the respective species according*

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Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

to their needs, i.e. social interaction, activity-related use of the space and appropriate stimuli and materials' (*Council of Europe, 1997*). Subsequently, Appendix A of the convention has been revised with new cage dimensions and space allocations for a range of species (*Council of Europe, 2006*).

The aim of this study was to evaluate the influence of different enrichment schemes on growth rate, water consumption, muscle strength and preference in rats, after items such as hides, nesting material, increased cage height and shelves had been introduced to the cage environment.

Materials and Methods

Housing conditions

Sixty male rats, NTac:SD (Taconic A/S, Lille Skensved, Denmark) arrived at the facility at the age of four weeks and were housed under one of the following three housing conditions after random distribution to the cages: Twenty rats were housed in groups of four in non-enriched type IV cages (non-enriched Type IV), 20 rats were housed in groups of four in enriched type IV cages (standard-enriched Type IV), and 20 rats were housed in groups of four in enriched Scantainer^{NOVO}-system cages (extra-enriched Type IV) (Figure 1). The standard-enrichment consisted of nesting material (Enviro-Dri®,Lillico Biotechnology, Surrey, UK), a biting stick (aspen brick, size M, Tapvei, Kortteinen, Finland) and hide (13x15x20 cm, black-transparent Plexiglas, Repsol, Denmark). The extra-enriched cage had a raised lid giving a total height of 32.5 cm, whereas the two other Type IV cages were equipped with normal flat lids (i.e. a total height of 18 cm). In the extra-enriched cage, there was a built-in plastic shelf (23x19 cm) in addition to the other standard-enrichment items. All cages were placed in a ventilated Scantainer^{NOVO} rack (Scanbur A/S, Karlslunde, Denmark) with 50 air changes per hour and the temperature at 24±1 °C and a humidity at 55±5%. All cages were solid bottom cages provided with bedding (aspen bedding, Tapvei, Kortteinen, Finland), food (Altromin 1324, Brogaarden, Gentofte, Denmark) and bottled tap water *ad libitum*. The cages were changed twice a week with new bedding and nesting material, whereas hide and biting stick were transferred to the new cage.

Preference study

After 12 weeks of housing under one of the conditions described above, eight animals (from two cages) from each condition were tested in a preference set-up to determine the dwelling time in each type of housing condition. The preference study was done in a Scantainer (Scanbur A/S, Karlslunde, Denmark) with the cages on a digital weighing

Figure 1. Three different housing conditions for laboratory rats. To the left the non-enriched Type IV cage, in the middle the standard-enriched Type IV cage (i.e. non-enriched condition supplemented with biting stick, nesting material and a hide), and to the right the extra-enriched Type IV cage (i.e. standard-enriched condition further supplemented with a shelf and a raised lid increasing the height of the cage from 18 cm to 32.5 cm).

system (EA6DCE-L, Sartorius AG, Goettingen, Germany), using a method described in a previous study (*Krohn & Hansen, 2001*). Data were collected for two full nights and two full days for all set-ups and the total dwelling time in each cage calculated. The following set-ups were analysed:

- Non-enriched animals (from non-enriched Type IV cage): Non-enriched cage vs. extra- enriched cage.
- Standard-enriched animals (from standard-enriched Type IV cage): Standard-enriched cage vs. extra-enriched cage.
- Extra-enriched animals (from extra-enriched Type IV cage): Non-enriched cage vs. extra-enriched cage, and standard-enriched cage vs. extra-enriched cage.

Home cage observations

Two cages from each housing condition were video-recorded for 12 hours in a 24-hour period constantly changing between three hours of recording followed by a three-hour pause, after which the video was analyzed by instantaneous sampling. For every sixth minute it was registered whether each animal was active or inactive, and whether the shelf in the extra-enriched cage was used.

The animals in each cage could not be individually identified on the video recordings, so the results are the mean of the four rats in the cage.

During the entire study period, the animals were weighed once a week, and the weekly water consumption for each cage was measured and calculated.

Muscle strength

The muscle strength of all the animals was tested at 18 weeks of age and at the end of the study. The test was done using the techniques described in a previous study (*Rivlin & Tator, 1977*). The platform used measured 52x38 cm with 14 cm high walls and was hinged on the rear end, and an electronic protractor was attached to the platform (Figure 2). The surface of the platform was made of rubber. The rat was placed on the platform facing the end opposite the hinged rear end. This end of the platform was then gently lifted. When the rat lost its grip on the surface and was sliding down from the platform, the angle of the platform was registered. Each rat was tested three times, and the mean value was calculated.

Figure 2. An inclined platform used for measuring muscle strength.

Statistical analysis

All data were tested for normal distribution by the use of Anderson-Darling normality test. The data for water consumption, weight gain and muscle strength were normally distributed and analyzed further with a one-way ANOVA (Minitab ver. 14.1, Minitab Inc., US). For the preference studies the results were statistically analysed by the use of a t-test (Minitab ver. 14.1, Minitab Inc, US), to determine whether the distribution between the left and the right cages was 50/50, as the data were normally distributed. The null-hypothesis was set as no effects of housing on the preference (a 50/50 distribution between the two cages) versus the alternative hyphothesis that an effect would be observable. As the animals were housed in only two cages for each condition, data were tested for an eventual cage factor as well.

No statistical tests were performed on the behavioral observations.

Results

The preference study showed that the rats spent more time in the standard-enriched cage compared to the non-enriched environment (Figure 3a), while there was no difference in dwelling time between standard-enriched and extra-enriched environment (Figure 3b). Rats in the standard-enriched environment had increased muscle strength compared to rats in non-enriched or extra-enriched environments (Figure 4),

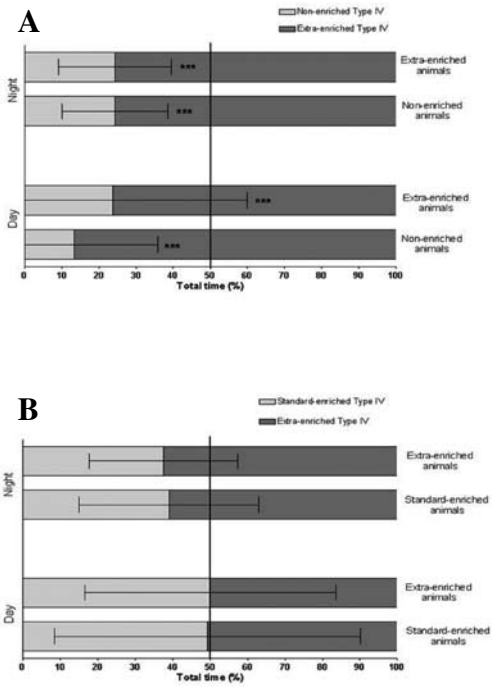

Figure 3. The results from the preference study. **a)** the preference for either a non-enriched cage or an extra-enriched cage in laboratory rats; **b)** the preference for either a standard-enriched cage or an extra-enriched cage of laboratory rats. The figure shows the distribution of dwelling time for rats used to be housed in either a non-enriched or extra-enriched cage when given the choice between a non-enriched cage or an extra-enriched cage for both day and night. The 50% distribution is marked with a bold line, and for each result the standard deviation is marked. ***: $p<0.001$.

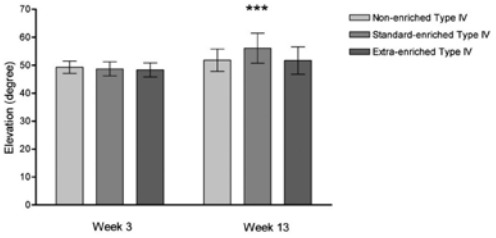

Figure 4. The muscle strength of laboratory rats under different housing conditions. The results represent the mean for all rats in each housing condition for week 3 and week 13 (Mean ± SD). ***: $p<0.001$).

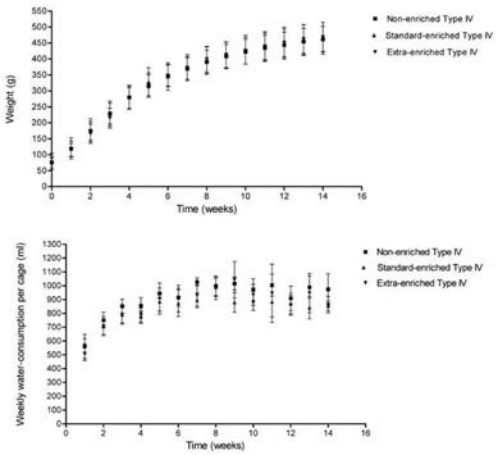

Figure 5. a) the weight gain of laboratory rats under different housing conditions. The results represent the mean value for the rats in each housing condition for every week in the experiment (Mean ± SD). P=0.9978; **b)** the weekly water consumption for laboratory rats in each housing condition. The results represent the mean value for each cage per week for each housing condition (Mean ± SD). P=0.2568.

but there were no differences in weight gain (Figure 5a) or water consumption (Figure 5b) in relation to the housing conditions.

From the behavioral observations, the rats in all three housing conditions were found to be active for 32.3% of the observations, and for the extra-enriched cage the shelf was used by at least one rat in 41.7% of the observations.

Discussion

The study demonstrated that rats spend more time in the extra-enriched cages compared to the non-enriched cages, whereas no differences in the dwelling time between the two types of enriched cages could be detected. When a cage is regarded as more aversive or more attractive compared to the other, a dwelling-time distribution as seen in the present study (Figure 3a) is common (*Krohn & Hansen, 2001*; *Krohn et al., 2003a*; *Krohn et al., 2003b*). Both cages may be regarded as attractive, but one may be more attractive, or both cages can be regarded as aversive, but one may be less aversive. It does not have to be aversive set-ups that are compared in the preference test. Therefore, a natural conclusion when comparing non-enriched cages against enriched ones would be that the rats prefer the enriched cages independent of their earlier housing history. Previous studies have also shown this preference for enriched cages (*Patterson-Kane et al., 2001*; *Townsend, 1997*). When comparing standard-enriched with extra-enriched cages, no preference, as indicated by dwelling times, for either of the cages can be seen (Figure 3b). The results show that some of the rats spend most of the time in the standard-enriched cage, whereas other rats spend most of the time in the extra-enriched cage, regardless of their earlier home-cage history, resulting in a mean dwelling-time for each cage of around 50%, as seen when the cages are regarded equally (*Krohn & Hansen, 2001*).

As behavioral data were collected at the cage level, the sample size is too small to permit a conclusion for general activity; however results indicate no difference in the general activity. Previous studies have show increased activity in the enriched cages (*Spangenberg et al., 2005*; *van der Harst, 2003*), but many factors, such as strain, age, cage size, etc besides enrichment may have an impact on the activity of the rats, and the way activity is defined may also differ between studies. The behavioral observations showed that the extra-enriched housed rats used the shelf extensively (more than 40% of the observations). Previous studies have shown that rats spend significant time with objects on which they can climb (*Williams et al., 2008*) and spend a significant amount of time with their heads raised above 17 cm if given the opportunity (*Büttner, 1993*), or performing more rearing when given the opportunity (*Hirsjärvi, 1994*). However, the cited studies were performed in cages without a shelf. Increasing cage height (from 20 to 91 cm) also eliminated stereotypic behavior in captive roof rats (*Callard et al., 2000*).

Increased muscle strength would have been expected for the extra-enriched housed rats as the raised lid and jumping on and off the shelf should have increased the muscles in the legs. The shelf is used intensively, but jumping on and off the shelf had not increased muscle strength. It is not possible to explain from the present study why only the standard-enriched housed rats had increased muscle strength.

In the present study it was not possible to show any differences in weight gain and water consumption for the three different housing conditions, which is similar to a mouse study in which no differences in weight gain were found (*Tsai et al., 2002*). Food consumption was not measured in the present study, as it is difficult to get a reliable result when rats are housed in ordinary cages where waste cannot be measured. But as no differences in the weight gain and the water consumption were found, no differences in food consumption would be expected , although final conclusions about this cannot be drawn. Other rat studies have shown altered weight gain for rats housed in an enriched environment. In one study an increase in weight gain was observed in the enriched environment (*van der Harst, 2003*), whereas another study showed reduced weight gain in enriched housed animals(*Spangenberg et al., 2005*). The latter were, however, housed in larger

cages. In our study, the cage size was the same for all groups. We chose this cage size because a Type IV cage was the only cage size for which shelf and raised lid were commercially available in a system which fits into a ventilated cabinet. Also, the type IV cage is large enough to give a good opportunity for enriching the cage whilst still leaving space for different activities for the animals. Furthermore, it should be noted that the shelf in the extra-enriched cage provides more than 400 cm^2 extra 'floor/rest area' compared to the other cages, even though it is not permitted to consider these extra square centimeters a legal part of in the minimum cage size.

The study demonstrated that rats spend more time in the extra-enriched cages compared to the non-enriched cages, whereas no differences in the dwelling time between the two types of enriched cages could be detected. When present in the cage, the built-in shelf was used extensively (more than 40% of the observations) although no specific preference for the extra-enriched cage was detected.

No differences in weight gain and water consumption were detected between rats in the three different housing conditions although there was a slight increase in the muscle strength for the standard-enriched housed rats.

We therefore conclude that we have not been able to show profound benefits from the commercially available extra-enriched system, compared to the standard-enriched system.

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