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# Standardisation of Environmental Enrichment for Laboratory Mice and Rats: Utilisation, Practicality and Variation in Experimental Results

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***Summary***

Rats and mice are the most commonly used species as laboratory animal models of diseases in biomedical research. Environmental factors such as cage size, number of cage mates and cage structure such as environmental enrichment can affect the physiology and behavioural development of laboratory animals and their well-being throughout their lives. Therefore compromising the animals' well-being due to inadequate environmental conditions would diminish the value of the research models. In order to improve laboratory animals' well-being and promote the quality of animal based biomedical research, it is fundamentally important that the environment of the animals meets the animals' species typical behavioural needs. Standardisation of environmental enrichment for laboratory rats and mice therefore should provide possibilities for the animals to engage in at least the essential behavioural needs such as social contact, nest building, exploring and foraging. There is a wide variety of environmental enrichment items commercially available for laboratory mice and rats. However, how these items are used by the animals, their practicality in the laboratory and whether these enrichments might lead to increased variation in experimental results have not been widely assessed. In this study, we implemented two standardised enrichment items (shelters, nesting materials) for rats and mice at different animal units. We instructed the animal care staff in monitoring the use of enrichment items by the animals by means of a daily score sheet system. The animal staff's viewpoint on practicality of the standardised enrichment program was assessed with a monthly score sheet survey. Also we assessed whether the enriched environment affected breeding results and contributed to an increase in variation of experimental data from several participating current studies. Our results show that the animals readily used the provided enrichment items. A slight increase in workload for the animal staff was reported. However, the overall judgement was mainly reported as good. Breeding results and variation in experimental data did not reveal differences as compared to data from previous housing and/or non enriched housing conditions. Overall, the results indicate that standard environmental enrichment that is species appropriate may enhance the animal's well-being without undesirable side effects on the experimental outcome and daily working routine of the animal care staff.

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***Introduction***

Environmental conditions such as housing and husbandry have an impact on the laboratory animal throughout its life, not only during the experiment itself, but also before and after the experiment. This has major implications for all contexts in which animals are maintained and cared for in the confined settings, because any compromising of the



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animals' well-being and causing them to develop abnormal behaviours would diminish their value as appropriate research models (*Garner 2005*). Environmental enrichment can be defined as any modification in the environment of captive animals that seeks to enhance their physical and psychological well-being by providing stimuli that meet their species-specific needs (*Newberry 1995, Baumans 2000*). In the Revision of the Guidelines on Accommodation and Care of Laboratory Animals of the Council of Europe (*ETS 123, 2006*) and the Revision of the European Council Directive 86/609/EEC (*2007*), a section on environmental enrichment has been included. Enriched environments release and structure species-typical behaviour, and more of the behavioural needs are met (*Fortmeyer 1982, Newberry 1995, Van de Weerd 1996*). It has also been shown that barren, restrictive and socially deprived housing conditions interfere with development and function of the brain, and with behaviour (*Mason 1991, Rosenzweig & Bennett 1996, Hall 1998, Callard et al. 2000, Mitsushima et al. 2001, Pham et al. 2002, Garner 2005*). Moreover, inadequate or unstructured conditions that do not meet the animal-specific comfort requirements may even encourage aggression and stereotypic behaviours (*Armstrong et al. 1998, Key & Hewett 2002, Risedal et al. 2002, Gebhart-Henrich et al. 2005*). However, scientists are concerned whether or not environmental enrichment would introduce more variability in experimental results. Standardisation increases the reproducibility and comparability of experiments. It aims to reduce unwanted variation caused by animal and environmental factors and to reduce the number of animals needed in experiments. Results from several relevant studies seem to indicate that the effects of enrichment on the variability in results depend on the parameter, type of enrichment and the animal strain used. (*Würbel 2001, Van de Weerd et al. 2002, Augustsson et al. 2003, Baumans 2005a,b, Bayne 2005, Würbel & Garner 2007*) Thus, housing conditions are highly relevant not only for the well- being of the laboratory animal, but also for the interpretation of the experimental results. Better knowledge of factors in the animals' environment that can influence their behaviour may lead to less variability in the experimental results and can thereby reduce the number of animals needed for experimental procedures. Standardisation of environmental enrichment, meeting the animal's species appropriate needs, might contribute to the animal's well-being, without increasing the variability in experimental results although the environment of the animal can never be standardized entirely (*see Crabbe et al. 1999, Nevison et al. 1999, Olsson & Dahlborn 2002, Wahlsten 2003, Van Loo et al. 2005, Würbel & Garner 2007*).

In this study, 4 different departments at the Karolinska Institute were recruited to participate in the assessment of a standardised environmental enrichment program. A standard enriched environment for laboratory animals should be simple and practical in order to motivate implementation by the animal care staff. However, the choice of the enrichment should be based on scientific evidence of the benefit for the animal. The ultimate goal of the study was to introduce a standardised environmental enrichment program (nesting material, shelter) for laboratory mice and rats that would promote the animals' species-typical behaviour. The selection of the environmental enrichment introduced was based on results from previous preference tests and behavioural studies (*Van de Weerd et al. 1994, 1997a, 1997b, 2002, Manser et al. 1998, Van Loo et al. 2005*) showing that tissues as nesting material and shelter are highly preferred by laboratory rodents. Standardizing the housing condition with preferred enrichments allows the animals more control over their environment, and fulfils some of their species-typical behavioural needs such as nest building, hiding and exploring. Increasing the animals' ability to control the environment may reduce stress as experienced by the animals, so they might be able to cope better with novel situations and consequently show a more uniform response in experiments (*for review see Bayne 2005, Garner 2005*). It was recently shown that good nest building activities and maintaining a well kept nest can be used as behavioural indica-

tors of well-being in laboratory mice that are subjected to invasive experimental procedures (*Arras, 2006*). For laboratory mice and rats, manipulating objects that can be transformed into nesting material and those that prompt the animals to engage in species-typical behaviours such as gnawing, shredding appear to be the most preferred enrichments in a long-term confined setting. These items have been documented to maintain over time their enrichment values for rats and mice (*for review see Olsson & Dahlborn 2002, Reinhardt & Reinhardt 2006*). The goal of introducing a standard enrichment program at a facility therefore, would be more effective and practicable when the items introduced do not lose their enrichment value with time as their novelty dissipates.

The objectives of this study were threefold. First we assessed whether and how the animals used the enrichments provided. The animal care staff was asked to monitor nest appearance, nest location and amount of enrichment items eaten or destroyed by means of simple daily score sheets. Second, the animal care staff's judgement on the standard enrichment introduced was assessed, in terms of daily work load and practicality. Furthermore, we determined whether the introduced enrichment affected breeding results. Third, we assessed whether the enrichment used changed variation in experimental results from different participating experiments.

***Materials and Methods***

The protocol for this study was reviewed and approved by The Southern Stockholm Animal Ethics Committee. All procedures involving live animals have been performed in accordance with Swedish National legislation and The Council of Europe Convention ETS123.

*Study 1: Use of Enrichment*

*Housing conditions*: The standard cage enrichment for the mouse (Photo 1) included social housing (3-6 animals/cage) in Macrolon Type III cage (800cm2) with wood chips bedding (B&K Universal, AB, Sweden), each cage provided with a Shepherd Shack/DesRes (SS/DR) shelter of egg-box type carton 15 x 9 x 6 cm (Des Res houses for mice, Lillico Biotechnology, Bodergaerd, Denmark) and two Kleenex® tissues (Kimberly Clark Corp. Sweden).

**Photo 1.** Standard enrichment for mice.

The standardised enrichment for rats (Photo 2) included social housing (4 animals/cage) in Macrolon Type IV cage (1800 cm2) with wood chips bedding and a Shepherd Shack/DesRes (SS/DR) (egg-box carton 21 x 20 x 10 cm, Des Res houses for rats, Lillico Biotechnology, Brogaarden, Denmark), an opaque yellow PVC tube (30 cm long, 12 cm diameter), folded paper strips as nesting material (Enviro-Dri, Scanbur, BK, Sweden), and two gnawing sticks (aspen wood, 1cm x 1cm x 5cm, Finn Tapvei, Finland).

**Photo 2.** Standard enrichment for rats.

*Animals and type of research*: Four different animal units at the Karolinska Institute on the Stockholm campus participated in the study:

1) Centre for Molecular Biology (CMB): Standardised enrichments were assessed in 3 different rooms with breeding animals for 3-12 week periods. Room I housed 40 cages of C57BL/6 male mice; room II included 40 cages of OP female & sterile male mice and 14 breeding cages of B6/CBA F1 female mice. Room III had 40 cages of B6/CBA F1 + CD1 mice. Separately, the animals were monitored for breeding results from enriched conditions (72 oviduct transfer pregnant B6CBAF1 + CD1 mice and 15 uterus transfer B6CBAF1 + CD1mice) and from non-enriched conditions (53 oviduct transfer B6CBAF1 + CD-1mice and 26 uterus transfer CD1 mice). The mice were housed 5 animals per cage.
2) Department of Neuroscience: Standardised enrichment was implemented in two studies. A morphine dependency study included 8 cages of male C57BL/6 and DARPP-32 knock-out mice (4 mice/cage); the study was carried out for 11 weeks. Other animals included in the assessment of variation of experimental results (study II) were 10 cages of adult male Sprague Dawley rats used in a spinal cord injury study for 6 weeks (4 rats/cage) and 4 cages (4 mice/cage) of BALB/c mice used in a study of running wheel effects on brain neurotrophin levels.
3) Microbiology and Tumour biology Centre (MTC): 6 cages of mice from a Pneumococcus infection/survival study that included the Toll-Like Receptors (TLRs), TLR6 & TLR4-deficient mice and wild type mice (wt) C57BL/6 (6 mice/cage). The study was carried out for one week.
4) Department of Pharmacology: 7 breeding cages of C57BL/6 mice for the period of 3 weeks. In two other rooms at the department of Pharmacology a different type of shelter (pizza box style shelter; Photo 3) was already in use at the time of this study (providing some environmental enrichment). Therefore in these two rooms, half of the cages were provided with pizza box shelters and the other half with the SS/DR shelter. 24 cages of AMPK-knock out mice (12 cages with pizza box; 12 cages with SS/DR) were monitored for 7 weeks. Six cages of AI Kongen I mice (3 cages with pizza box; 3 with SS/DR) were monitored for 13 weeks. For breeding results, the numbers of pups born were recorded from 32 AMPK knock-out pregnant mice; 16 were housed in standard enriched condition and 16 in non-enriched condition.

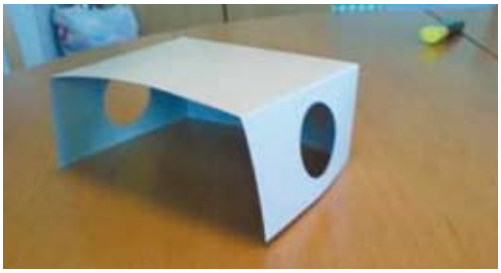

**Photo 3.** Pizza box style enrichment.

*Data collection*: Each animal unit was provided with the daily checklist protocol. Before the study begun the researchers met with the animal care staff from each animal unit and instructions were given on how to fill out the checklists .Parameters measured:

1) Use of enrichment in each cage was recorded on a daily basis excluding weekends. The following parameters were scored with check marks:
   - A Nest appearance: defined as the final result of the nest building activities using the provided tissue materials to make and to keep a nest. The nest is the place where the animals sleep, rest and/or nurse pups. Score as good, some what or none/bad.
   - B Nest location: the place inside the home cage where the nest is built in relation to the shelter. Score as inside shelter, beside shelter or away from shelter.
   - C Place of nesting materials: the tissues arrangement within the cage in spatial relation to the shelter. Score as inside, beside or away from the shelter.

D Amount of enrichment items eaten (2 tissues, 1 SS/DR shelter): visual estimation of the quantity of the provided enrichment items being eaten. Score as none, some or a lot.

2) Practicality (control of animals, cleaning of cages), daily work load of animal care staff and their overall judgement of the standard enrichment introduced were assessed using a monthly checklist.
3) Breeding results (total number of pups born/dam) during the study period were obtained from records kept at the participating animal units.

*Data analysis*: The results of use of the provided enrichment by the animals recorded by daily checklists were summarized separately according to rooms and departments. Data on (A) Nest appearance, (B) Nest location, (C) Place of nesting material, (D) Enriched items eaten were summarized by week, cage and then averaged over the experimental period. The daily (5 days-week) assessments by checking the three possible choices of each parameter on the score sheet (see appendix 1) were transformed into quantifiable fractions of 100 percent value of occurrence for each week.

The monthly scorings of practicality and work load based on the 3 possible choices for each parameter on the survey checklist were transformed into percent of occurrence over the four month period of the standard enrichment program.

Due to the short period of the study (1-13 weeks), comparison could only be made on the mean number of pups born/dam between the mice housed in standard enriched environment and those in non-enriched housing. Student's t-test was used to determine whether enriched and non-enriched mice differed significantly in the number of pups born per dam.

*Study 2: Variation in experimental results*

*Housing condition:* Two housing conditions were used: standard enriched (same as in study 1) and non-enriched conditions consisting of socially housed animals (3-6 mice/cage, 4 rats /cage) with wood chips bedding only.

Participating researchers were asked to provide individual data of animals in each study and compare them with either non-enriched groups or groups provided with the previously used enrichment (pizza box style shelter). Group mean values of the dependent variables measured were calculated. To determine variation within parameters measured we used the Mean Absolute Deviation (MAD) method, as the absolute deviations can be calculated for each individual observation whereas e.g. a standard deviation gives one value for the whole group. This means that the dispersion in the individual observations can be analysed in the same way as the individual observations themselves (*Van de Weerd et al., 2002, Augustsson et al, 2003*).

To analyse possible differences in MAD, the following formula was applied:

Group observations were calculated as X*mean*. The observation per individual was indicated as $X_{i,}$ the absolute deviation was calculated as:

$$| X_i - Xmean |$$

Data collected from separate experiments were analysed for differences in both mean and MAD variation. Data samples from mice and rats housed in standard enriched and non-enriched conditions in the 3 separate studies were assessed and the following dependent variables were subjected to analysis for mean and MAD variation: Survival, body weight, open field behaviours, BDNF (brain derived neurotrophic factor) levels. Statistic analyses were conducted with StatView for Windows (version 5.01). The behaviour data and the levels of BDNF were analysed by analysis of variance (ANOVA) using 2 × 3 (housing × treatment) randomized block design. Statistical significance was set at a probability level of $P < 0.05$ for all tests. Post-hoc test (Fisher's Post-hoc test) was used when ANOVA showed significance.

To analyze for possible effect of the housing conditions, data from open field test and brain neurotrophins were subjected to analysis of variance (ANOVA), data of body weight were analyzed by ANOVA with repeated measures over time.

***Results study I***

*Use of the enrichment*

*MTC:* The results from C57BL/6 wild-type and TLR deficient mice indicated that good nests were built and located inside the SS/DR shelter in 100% of the cases. Occurrence of tissues placed inside the SS/DR shelter was 88%, and 12% for tissues placed outside but close to the shelter. The incidence of some amount of tissue and shelter materials eaten over the one week period was about 30% (Table 1).

*Neuroscience:* Results from animal care staff's scoring of nest appearance revealed good nest building activities in more than 80% of the cases over the 11 week period. The incidence of tissues and shelter materials eaten was about 20%. Both the place where nesting materials were put and the nest location were 87% and 91% of the time respectively inside the provided SS/DR shelter (Table 1).

Results for use of enrichment from 4 cages of adult Sprague Dawley rats over a 5 week period are shown in Table 2. Good nest appearance occurred in 98% of the cases, and nests were located inside the PVC tube and inside the SS/DR shelter at 70% and 10% respectively over the 5 weeks. 17% of rats ate some of their nesting and shelter materials.

*Pharmacology:* Table 1 shows results from 7 cages of C57BL/6 mice monitored over 3 weeks. Good nests were built in 91% of the cases. The nests were made with the provided tissues inside, next to and

**Table 1.** Use of standard enrichments by laboratory mice

| Animal facilities | MTC | Neuroscience | Pharmacology | CMB |
|---|---|---|---|---|
| **Nest appearance** | | | | |
| Good | 100 | 87 | 91 | 90 |
| Fair | 0 | 13 | 9 | 8 |
| No or bad nest | 0 | 0 | 0 | 2 |
| **Nest location** | | | | |
| Inside shelter | 100 | 91 | 73 | 93 |
| Beside shelter | 0 | 4 | 17 | 1 |
| Away from shelter | 0 | 5 | 10 | 6 |
| **Place of tissues** | | | | |
| Inside shelter | 88 | 87 | 68 | 83 |
| Beside shelter | 12 | 6 | 22 | 10 |
| Away from shelter | 0 | 7 | 10 | 6 |
| **Enrichment materials eaten** | | | | |
| None | 70 | 78 | 44 | 62 |
| Some | 30 | 22 | 56 | 37 |
| A lot | 0 | 0 | 0 | 1 |

The table summarises results of mean percentages of occurrences in parameters monitored by daily score sheet at the four different animal facilities. MTC (6 cages); Neuroscience (8 cages); Pharmacology (7 cages), CMB (120 cages).

**Table 2.** Use of enrichments by rats at Dept of Neuroscience

| | |
|---|---|
| **Nest appearance** | |
| Good | 98,4 |
| Some | 1,6 |
| None | 0 |
| **Nest location** | |
| Inside SS/DR shelter | 10,4 |
| Inside PVC tube | 70,4 |
| Away from SS/DR or PVC | 19,2 |
| **Place of nesting materials** | |
| Inside shelter | 27,2 |
| Inside PVC tube | 48,8 |
| Away from SS/DR or PVC | 21,6 |
| **Enrichment materials eaten** | |
| None | 83,2 |
| Some | 16,8 |
| A lot | 0 |

The table summarises results of mean percentages of the use of enrichments as monitored in rats by daily score sheet. (Department of Neuroscience: 4 cages).

away from the SS/DR shelter in 68%, 22 and 10 % of the cases respectively. Location of the nest was recorded inside the SS/DR shelter 73%, beside the shelter 17% and away from the shelter 10% of the times. Incidence over the 3 week period of some enrichment materials eaten was 56%.

The AMPK knock-out mice in room 1 showed good/fair nest appearance in cages with SS/DR shelter 92 % of the time versus 90 % occurrence in cages with pizza box type shelter. The nest locations occurred about 71 % versus 93 % inside the SS/DR and pizza box type shelters respectively. Similarly about 68% compared to 94% of tissues placed inside and 20 % versus 4 % were placed near to the respective shelters. Incidences of some enrichment materials eaten were 40 % in cages with SS/DR and 19 % in cages with pizza box shelters. In room 2 with AI-Kongen mice, nest locations were recorded to occur mainly inside the shelters, 94% for SS/DR and 99% with pizza box type. However, unlike room 1 while tissues were placed mainly inside the shelters , 94% in SS/DR versus 99% in pizza box, good nest appearance was low (4% and 13%) and the occurrence of some enrichment materials eaten was high (62% and 49%) in cages with SS/DR or pizza box respectively.

*CMB:* Overall results from the CMB department showed that good nest building in the cages was scored at 90%, nest location inside the shelter was recorded at 93% a nd tissues placed inside the provided shelters was scored at 83%. The frequency recorded for the parameter of "some amount of enrichment items eaten" was 37% (Table 1).

*Practicality and daily workload*

The animal care staff's judgements of the introduced standard enrichment can be summarised as followed: The staff rated "Control of animals" as easy or normal about 83%, as more than normal 0%, and as difficult or much more than normal about 17% of the times. "Cleaning of cage" was reported as normal (100%) over the entire period of the study, while the parameter of "Daily workload" was reported at the frequency of 100% to be more than normal (Fig 1). Results of the overall judgement of the standard enrichment program indicated that the animal care staff judged the standard enrichment as good about 67% of the time, as equally fair 17% and bad 17% of the time (Fig 2).

*Breeding results*

Results of breeding of mice at two different animal units are shown in Table 3. There were no significant differences in the mean number of pups born per dam from standard enriched condition compared to previous non-enriched condition.

***Results study II***

*Variation in experimental results*

*Survival scores and variation in survival scores:* Table 4 shows mean survival score in hours and their

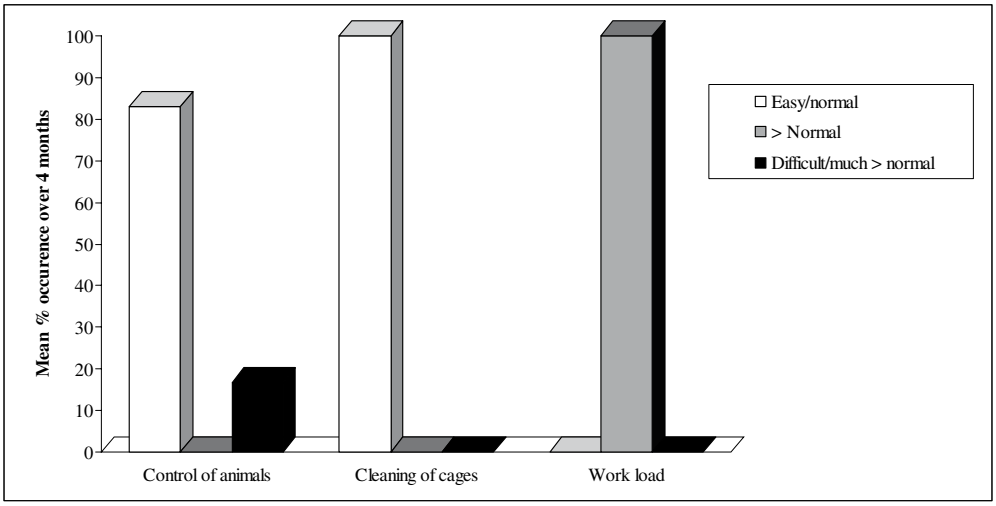


**Figure 1.** Control of animals, cage cleaning and daily workload rating of the standard enrichment program by the animal staff.

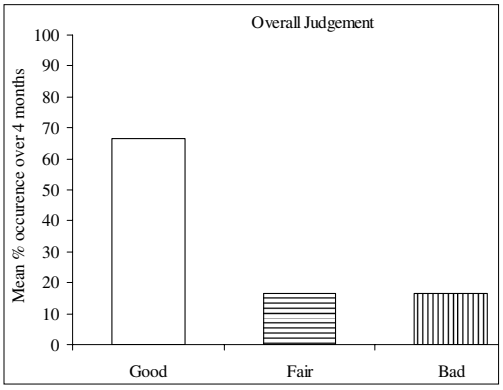


**Figure 2.** Overall judgement of the standard enrichment program by the animal staff.

mean absolute deviations of the different groups of mice (TLR deficient and their control C57BL/6 wild-type) in a pneumococcal infection study. There was neither a significant difference in the survival parameter nor in the MAD between the groups housed in the enriched and non-enriched conditions although a trend was shown in enriched animals for a slightly higher variation than non-enriched animals.

**Table 3.** Standard enrichment breeding results

| Mice (Breeding unit) | Oviduct transfer (CMB) | Uterus transfer (CMB) | AMPK knock-out mice (Pharmacology) |
|---|---|---|---|
| Enriched condition | 5,14 (n=72) | 4,07 (n=15) | 4,88 (n=16) |
| Non-Enriched condition | 5,32 (n=53) | 4,15 (n=26) | 6,06 (n=16) |

Average number of pups born per dam (n = number of pregnant dams) housed in standard enriched or non enriched conditions from 3 different breeding rooms.

*Distance moved, speed of locomotion in open-field test and variations in distance moved and speed of locomotion:* The ANOVA shows no significant differences for housing conditions in distance moved and speed of moving or their MADs in the open-field test (*data adapted from Pham et al. 2005*) ( Table 5).

**Table 4.** Mean values of survival time after nasal pneumococcal infection and their Mean Absolute Deviations (MAD) for mice in the enriched and non-enriched housing conditions

| Groups | Enriched | | Non-enriched | |
|---|---|---|---|---|
| **Mice** | **Mean** | **MAD** | **Mean** | **MAD** |
| wt+ | 115,60 | 41,92 | 67,20 | 5,76 |
| wt- | 121,20 | 56,16 | 67,20 | 5,76 |
| Tlr4+ | 121,60 | 55,68 | 112,80 | 44,16 |
| Tlr4- | 168,00 | 0,00 | 151,20 | 26,88 |
| Tlr6+ | 131,40 | 43,92 | 141,60 | 42,24 |
| tlr6- | 111,20 | 45,44 | 127,20 | 32,64 |

**Table 5.** Mean values of open field test and their Mean Absolute Deviations (MAD) from mice housed in enriched and non-enriched conditions.

| Parameters | | Enriched | Non-enriched |
|---|---|---|---|
| Distance moved | Mean | 1701,34 | 1302,56 |
| | MAD | 287,23 | 348,12 |
| Velocity | Mean | 4,73 | 3,62 |
| | MAD | 0,80 | 0,97 |

*Brain derived neurotrophic factor* (*BDNF) levels in different brain regions and variation in BDNF levels:* Results of BDNF levels from mice housed in standard enriched and non-enriched conditions are shown in Figure 3. ANOVA indicated no housing effect on BDNF levels in brain regions assessed. There was also no significant difference in the MAD (see bottom figure) of BDNF levels between the enriched and non-enriched group housed mice (*data adapted from Zhu et al. 2006*).

*Body weight and variation in body weight:* Table 6 shows the mean weekly body weight and their MADs for the rats in each housing conditions, enriched and non-enriched. ANOVA with repeated measures over weeks indicated no significant housing effect. There was a significant time effect and an interaction effect between housing x time ($p < 0.05$). Over the 6 weeks of observation the non-enriched rats gained more weight than the rats housed in enriched condition. The MAD of body weight over time between the two housing conditions did not show a significant difference (*data adapted from Erschbamer et al. 2006*).

**Table 6.** Mean body weight and mean absolute deviations (MAD) of rats housed in enriched and non-enriched conditions.

| Parameters | | Enriched | Non-enriched |
|---|---|---|---|
| Bodyweight | Mean | 19,88 | 20,02 |
| | MAD | 0.78 | 0.87 |

***Discussion***

This project evaluated the use of a standardised enriched housing condition for laboratory rodents (rats and mice) in 4 different animal units at the Karolinska Institute. The enrichment used focused on the species -specific needs of nest building, hiding and exploration. The main goal of the project was to assess whether and how the animals use the enrichments provided. Second, to evaluate the practicality for implementing the standardised enrichment from the animal care staff's points of view and the third goal of the study was to assess whether the enrichment introduced would result in an increased variation in experimental data as compared to data obtained from animals housed in non-enriched environment or housed with the previously used type of enrichment.

The results of the use of enrichment from the four different animal units clearly showed that all animals used the nesting materials to build a nest. Daily observations using a checklist protocol by the animal care staff further confirmed that mice have a strong preference for tissues to build nests (*Van de Weerd et al. 1997a, 1997b, Olsson & Dahlborn 2002*). Results from all four animal units indi-

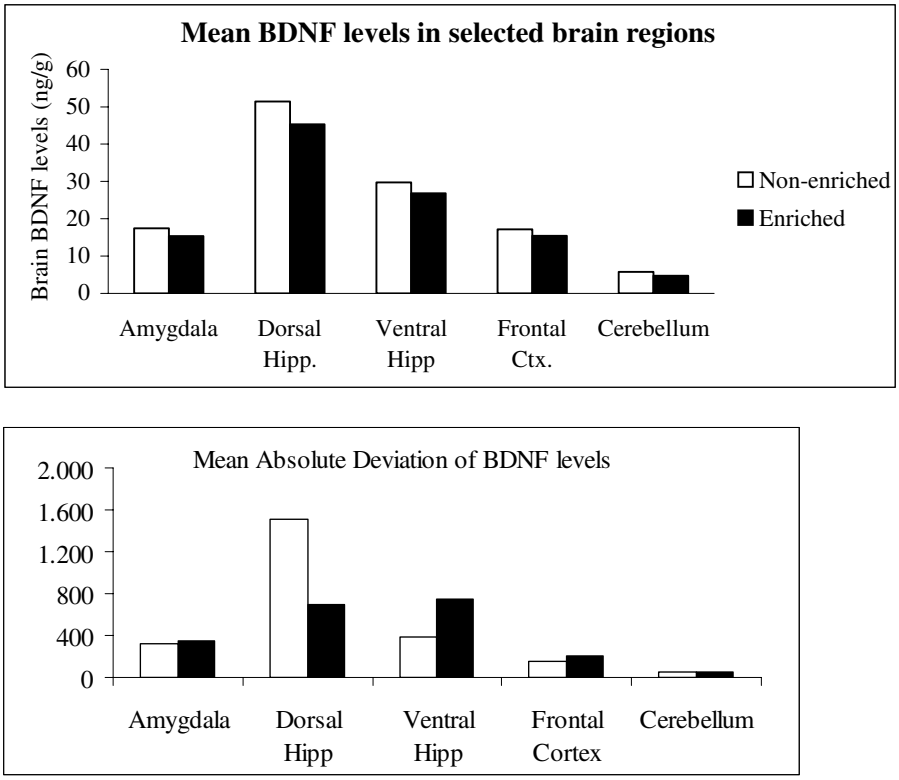


**Figure 3.** Mean BDNF (brain-derived neutrophic factor) levels in selected brain regions of mice housed in enriched and non-enriched conditions (top), and their mean absolute deviations (MAD) are shown in the bottom graph. Hipp=Hippocampus, Ctx=Cortex.

cated that the majority of animals included in this study built good nests. Good nest appearance was recorded in 80% or higher of all cases during the study period ranging from 1 to over 13 weeks from all housing units with the exception of one room at the department of Pharmacology having less than 20% occurrence of good nest appearance. In this case, where less than 20% of good nest appearance was recorded, the scoring of some amount of enrichment items eaten was higher. That means that in this room, where despite a high incidence of nest locations occurring inside the SS/DR and pizza box type shelters and where the Kleenex tissues were placed most often inside the provided shelters, the staff's subjective recording of good nest appearance was low. It seemed that although recording of good nest appearance was higher in cages with pizza box type shelter than SS/DR shelter, the percentage of enrichment items eaten was inversely correlated with the scoring of nest appearance, such that lower percentages of enriched items eaten or destroyed always paralleled higher percentages of good nest appearance as recorded by the animal care staff. Thus, the determinant factor for the animal care staff to score a nest as good was based on how intact the shelter remained and not on how the animals used the enrichment items.

Our results further support other findings of the importance for mice and rats to build their own nests according to their preference and species typical

needs (*Van de Weerd et al. 1997b, 1998*). Concerning the location where the animals place and disperse the provided tissues material, results from all units revealed that the tissues were most often found inside the shelters. This was further confirmed by the observation that mice would drag and shred the soft tissues to build a nest (*Van de Weerd et al. 1994, 1996, 1997a, 1998, 2002, Van Loo et al. 2005*). Rats, mice and hamsters readily work for appropriate nesting material such as soft tissues or paper material, (*Jansen et al. 1969, Oley & Slotnick 1970, Roper 1975, Collier et al. 1990, Manser et al. 1998*) indicating that nesting material is an essential resource which should always be supplied in any standard enrichment protocol. In this study nest location was defined in spatial relation to the provided shelter. The results of nest location indicated that most animals built their nest inside the shelter. Even in the room where good nest appearance was scored lower than 40%, the location of the nest was at least in 80% of the cases or higher inside the SS/DR or the pizza box shelters. The high occurrence of nest location inside the SS/DR shelter further confirmed previous study results showing that mice have a higher preference to build their nest inside the SS/DR shelter compared to another type of shelter (*Van Loo et al. 2005*), whereas rats showed a preference for the PVC tubes. Although the higher percentage of enriched materials eaten scored by the animal care staff might influence their personal bias in judging nest appearance and their preference for indestructible enrichments, prefabricated indestructible structures such as plastic shelters are not a mouse's choice for nesting and sleeping (*Van Loo et al. 2005*). In this study, preference for different types of shelter was not assessed. However, at the department of Pharmacology another type of shelter, the "pizza box" type was already in use when this study started. Therefore also the use of the two different types of shelter for nesting, nest location and place of nesting material could be compared in two separated rooms. In one room good nest appearance was scored at a higher percentage with the pizza box than the SS/DR shelters (97% versus 87%). This could be due to the fact that these mice were already used to the pizza box. The score for some amount of enrichment items eaten was higher with the SS/DR shelter, (6 % in cages with pizza box versus 35% in cages with SS/DR shelters). This was probably due to the softer material of the SS/DR than the pizza box shelter. In another room the occurrence of good nest appearance was low in both cage conditions (furnished with pizza box or SS/DR shelters), but the nest was located in more than 60% of the cases inside the shelters. Place of nesting materials was recorded as inside the SS/DR shelter more than 60%, and inside the pizza box more than 80% over the period of 13 weeks. Thus when appropriate shelter and nesting materials are provided, the mice will use these items to build good nests inside the shelter. This further confirmed previous findings that mice have a strong drive to construct their own nests. Soft paper nesting material is, therefore, more important to them than a pre-formed shelter (*Van Loo et al. 2005, Reinhardt & Reinhardt 2006*).

Workload was reported to increase in general during the study period, and control of animals was scored half of the time as normal and half of the time as difficult, due to less visibility of the animals and thereby fewer possibilities for observation without taking animals out of the cage. Cage cleaning was reported 100% the same as before. However, the majority of animal care staff scored the introduced enrichment as good. They reported the enrichment to be very useful for the animals. Breeding results from the department of Pharmacology and CMB showed no difference in number of pups born per dam housed with enrichment and those housed in non-enriched cages. Thus this type of enrichment did not seem to affect the fertility rate. A longer period of assessment and larger breeding units would be useful to confirm results from this study.

The type of environmental enrichment used in the participated studies did not influence variability for any of the parameters measured. Applying the method of comparing MADs, our evaluation of data variability that may be due to enriched housing condition indicated similar results to other previ-

ous studies (*Van de Weerd et al. 2002*). Furthermore in some parameters (open field test) the variability seemed to be less, although not significant, in data from enriched housed mice than from non-enriched housed mice; while in the parameter of survival time the data variability showed a trend to be higher in enriched than non-enriched housed mice. Other animal studies using different methods such as the coefficient of variation to compare data variability between enriched and non-enriched housing conditions also found increased, decreased or similar in data variations (*Eskola et al. 1999, Kuhnen 1999, Tsai et al. 2002, Wolfer et al. 2004*). From our study, we can conclude that the standard environmental enrichment used did not affect the variability in results of the experiments. However, we should emphasize that variability in results will depend on the type of parameters measured, the strain of animals and the type of enrichment used. Environmental enrichment used in neuroscience studies (large cages and frequent changing of enrichment items) with the aim to induce changes in the brain and in behaviour essentially will have a different impact on the animal than a simpler type of enrichment aiming at meeting some of the essential species-typical needs of laboratory mice and rats such as nesting, hiding and exploration.

In conclusion it can be said that the standard environmental enrichment used in this study was readily used by nearly all animals. Although a slight increase in workload for the animal care staff is reported, the overall assessment was rated as good in most cases.

Breeding results in mice were not affected by the introduced enrichment at the two departments in this study. The results indicate that the introduction of this type of a "standard" environmental enrichment enhances the animal's well-being without undesirable side effects on the experimental outcome and the daily working routine of the animal care staff.

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