

**BEHAVIOURAL PATTERNS OF LABORATORY MICE HOUSED UNDER  
ENRICHED AND STANDARD HOUSING CONDITIONS**

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### SUMMARY

*A newly developed behaviour registration system LABORAS was used to register automatically the behavioural patterns of mice during 24 h. Prior to this test the mice were housed for six weeks under either enriched or standard conditions. Housing conditions during the test were similar to those before the test. We have tested whether the behavioural patterns of animals in enriched housing conditions differ from their counterparts in non-enriched environments, in particular whether a difference in time budget or in the circadian rhythm of behaviour exists.*

*With the LABORAS behaviour registration system no effects of environmental enrichment on the circadian rhythm of behaviour of the mice were detected. However, differences in time budget of mice housed in the two different environments were established as well as differences in behaviour between strains.*

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## INTRODUCTION

Environmental enrichment is the process of improving the living conditions of laboratory animals with the goal to enhance their welfare. By introducing enrichment into the animals' environments they are able to express more of their species-specific behavioural repertoire (Chamove 1989a; Van de Weerd & Baumans 1995). As a consequence, the behavioural patterns of animals housed under enriched housing conditions will differ from their counterparts in non-enriched environments which are only able to perform a smaller part of their behavioural repertoire. Several studies have described differences in behaviour between animals housed in enriched or standard environments. Rabbits in cages enriched with objects showed an increase in active behaviour (consisting mainly of interactions with the enrichment objects) as compared to rabbits housed without enrichment who spent 80% of their time being inactive (Brooks et al 1993; Huls et al 1991). Rats provided with gnawing objects also showed an increase in active behaviour and a decrease in fighting (Orok-Edem & Key 1994). A nest box provided to mice increased explorative behaviour for a short period of time and changed space usage for a longer period of time: the mice slept in the nest box (Van Loo et al 1996).

Thus, environmental enrichment may change the time spent on different behavioural categories (time budget). It is also possible that the distribution of behaviour in time (circadian rhythm) changes as a consequence of environmental enrichment. Mice have clear circadian rhythms in their behaviour. They are most active during the night, with an activity peak shortly after the onset of the dark period. Thereafter periods of rest and activity alternate. Before dawn they show another, less prominent activity peak. During the day they mostly sleep (Schlingmann et al submitted; Van Oortmerssen 1971; Weinert 1994-96).

Evaluation of the effects of environmental enrichment on the behavioural patterns of laboratory mice requires behavioural observations during prolonged periods of time, using e.g. time lapse video recording techniques. Acquiring and analysing this information is rather time consuming and this can be reduced with the use of automated behaviour registration systems. Most behaviour registration systems only measure overall activity (e.g. Barclay et al 1988; Minematsu et al 1991; Young et al 1993). Whereas it might be important to register more behaviours at the same time. Some behaviours might increase while at the same time others might decrease, in which case overall activity remains the same and differences are not detected (Baumans et al in press).

In the present study the newly developed system, LABORAS (Bulthuis et al in press; Van de Weerd et al in preparation) was used for the automated registration of behaviour in six different categories during prolonged periods of

time. Mice of an outbred and an inbred strain were used in order to study possible influences of environmental enrichment on circadian rhythms and time budgets.

## ANIMALS AND METHODS

### *Animals and housing conditions*

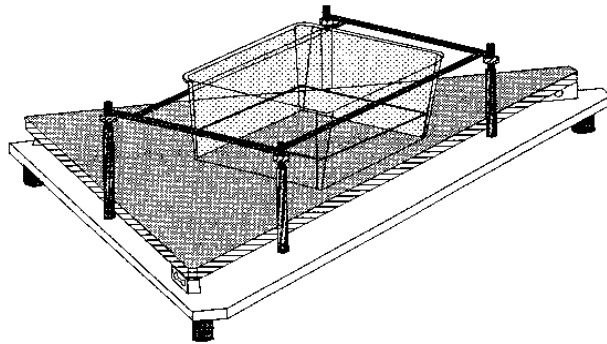
A total of 48 naive male mice of an inbred strain (BALB/cAaNCrIBR, n=24) and an outbred strain (CrI:NMRI BR, n=24) was used. They were bred without nesting material. At the start of the experiment they were three weeks of age. The mice were housed in a room with controlled photo-period (lights on 7.00-19.00 h, white light 355 lux (at 1 m above the floor), relative humidity ( $55 \pm 5$  %) and temperature ( $22 \pm 1$  °C), a radio played 24 h a day. The mice were housed per strain in groups of four animals in wire topped Macrolon type III cages (840 cm<sup>2</sup>, UNO Roestvaststaal, Zevenaar, The Netherlands) provided with sawdust (Lignocel S 8/15, Rettenmaier & Söhne, Ellwangen-Holzühle, Germany). Tap water and food-pellets (RMH-TM, Hope Farms, Woerden, The Netherlands) were provided ad libitum.

Per strain half the number of mice (three groups) were randomly allocated to either standard or enriched housing conditions for a period of six weeks. Standard cages contained only sawdust bedding (Lignocel S 8/15). Enriched cages were provided with sawdust bedding and the following objects: a nest box, consisting of a plastic PVC tube (length: 11 cm,  $\varnothing$  7 cm), big enough to give shelter to all animals of a group, nesting material (1 tissue, 25x44 cm, Kim wipers, Kimberly-Clark Corporation<sup>®</sup>, Veenendaal, The Netherlands) and a metal climbing grid, vertically attached to the cage lid (size: 16x10 cm, mesh size: 5x5 mm). The location of the objects remained the same throughout the experimental period. The tissues were renewed with weekly cage cleaning. Four days before the start of a test the mice were brought in their cages to the test room to acclimatise.

### *The LABORAS test system*

The LABORAS system is a fully automated device for the recording of behaviour of individually housed mice or rats. The system consists of a sensing platform (Figure 1) which is positioned on two orthogonally placed sensors and a third fixed point. A Macrolon cage (type II or III) is placed on this sensing platform. Each sensor transforms the mechanical vibrations caused by the movements of the animal into electrical signals, which are stored on a computer. Each movement has its own unique frequency and amplitude pattern and thus separate behavioural categories can be distinguished and classified by the computer. The

upper part of the cage (with the cage lid) is separated from the lower part of the sensing platform to detect climbing behaviour, because in this way a signal is not evoked by climbing behaviour (either on the cage lid or climbing grid).



**Figure 1** *The LABORAS behaviour registration system. Sensing platform with cage.*

#### *Procedure*

A mouse was placed in a (clean) Macrolon type III cage (840 cm<sup>2</sup>) similar to its home cage (either enriched or standard). Enrichment was provided during the test, because otherwise the absence of enrichment might evoke behaviours (such as increased exploration) and the behavioural patterns will not be representative for a mouse in an enriched cage. During 24 h the behaviour of a mouse was

recorded. Each test started between 14.00 and 16.00 h. Testing lasted for three weeks. Per week four groups of mice were tested in random order, the four mice of a group were tested at the same time, each on one sensing platform.

The LABORAS system distinguishes the following behavioural categories (Based on the ethogram in Schlingmann et al submitted).

resting =

movements are absent while the animal is in a sitting or lying position. Very short movements (e.g. turning over while sleeping) are not considered as an interruption.

locomotion =

activities such as walking, running, or jumping.

climbing =

climbing and hanging on the bars of the wire cage lid or food hopper or on the climbing grid in the enriched cages. While the animal is climbing or hanging, the hind legs or tail may touch the floor or side wall of the cage.

grooming =

the mouse is shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals.

eating =

the animal eats food pellets while standing upright, gripping the bars of the food hopper with its front paws, and gnawing the food between the bars. It also includes gnawing a particle of food clasped between the front paws.

drinking =

the animal stands upright to lick water from the water bottle.

undefined =

all behaviour which is not classified in one of the previous categories.

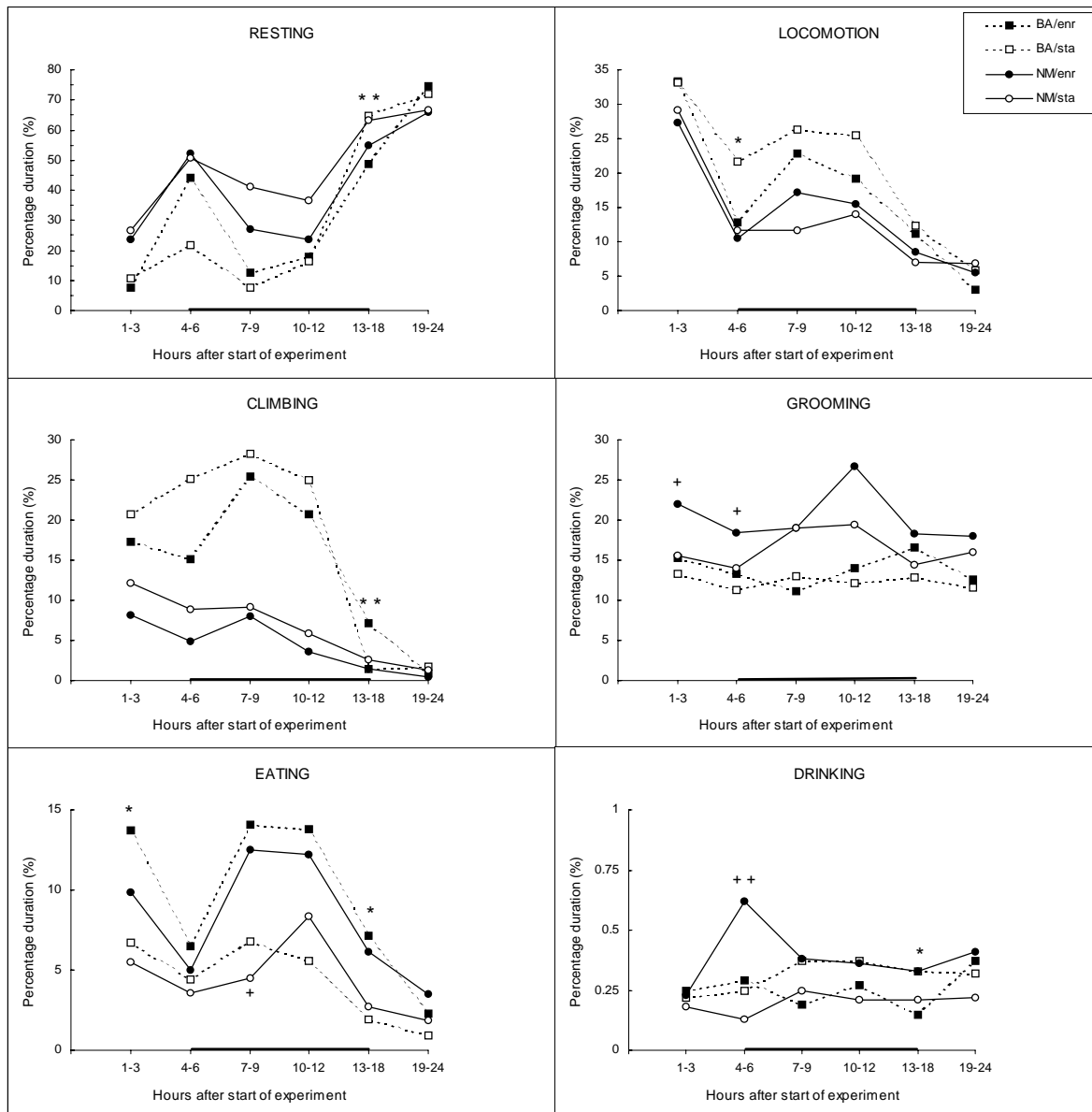
### *Statistical analyses*

Data were analysed using an ANOVA, to detect possible differences between the strains and housing conditions. The 24 h of the experiment were divided in six time periods: 1-3 , 4-6, 7-9, 10-12, 13-18, 19-24 h after the start of the experiment. Per time period the relative time spent on each behavioural category was calculated and analysed. A group of mice is treated as an experimental unit, to rule out influences of home cage and testing time. The level of statistical significance was pre-set at  $P < 0.05$

## RESULTS

Figure 2 shows the results of the 24 h behaviour registration. Per time period, the

mean time spent on each of six behavioural categories are shown per strain and housing system. The data of the category 'undefined' are not shown (overall less than 10%).



**Figure 2** Results of LABORAS behaviour registration system. Behavioural patterns of male mice of two strains (BALB/c and NMRI, N=48) housed under enriched or standard conditions are shown. Relative mean time spent on behaviour in six different categories during 24 h of testing. \*  $p < 0.05$ , \*\*  $P < 0.01$ , significant difference between housing conditions in BALB/c mice. +  $p < 0.05$ , ++  $P < 0.01$ , significant difference between housing conditions in NMRI mice. Lights went out in the 4-6 h period and on again in the 13-18 h period.

Overall, BALB/c mice from enriched conditions spent more time on eating (1-24 h,  $P < 0.05$ , also significant in 1-3 h period,  $P < 0.05$  and 13-18 h period,  $P < 0.05$ ). In the 4-6 h period standard housed mice spent more time on locomotion

( $P < 0.05$ ). The main differences between mice from the two housing conditions in this strain were found in the 13-18 h time period (enriched housed mice: more climbing,  $P < 0.01$  and eating,  $P < 0.05$  and standard housed mice, more sleeping,  $P < 0.01$  and drinking,  $P < 0.05$ ).

Overall, NMRI mice from enriched conditions showed more grooming (1-24 h,  $P < 0.05$ , also significant in the 1-3 and 4-6 period, both  $P < 0.05$ ) and drinking (1-24 h,  $P < 0.05$ , also significant in the 4-6 h period,  $P < 0.01$ ). Enriched housed mice also spent more time on eating in the 7-9 h period ( $P < 0.05$ ). NMRI mice from standard housing conditions spent overall more time on climbing (1-24 h,  $P < 0.05$ ) and on sleeping (1-24 h,  $P < 0.05$ ).

Overall significant differences between the strains were found, BALB/c mice spent more time on climbing (1-24 h,  $P < 0.001$ ) and locomotion (1-24 h,  $P < 0.01$ ) than NMRI mice, whereas NMRI mice spent more time on grooming (1-24 h,  $P < 0.001$ ) and resting (1-24 h,  $P < 0.01$ ) compared to BALB/c mice.

## DISCUSSION

The results of the 24 h behaviour observations showed similar overall behavioural patterns in both strains. High levels of activity were observed in the first 3 h (high levels of locomotion and climbing). After the introduction of mice in new cages (e.g. after cage cleaning) high levels of activity associated with exploration are often seen (Saibaba et al 1995). During the dark period, the mice were also active and showed high levels of eating behaviour. When the lights turned on again (13-18 h period), resting increased. Grooming was fairly constant during the whole 24 h period. These behavioural patterns are consistent with circadian rhythms of mice as found by other authors (Büttner 1991; Minematsu et al 1991; Schlingmann et al submitted; Weinert 1994-96). Overall, the results showed that the circadian rhythms in behaviour were not influenced by environmental enrichment. However, housing effects were found in both strains indicating that mice housed in enriched environments had different time budgets than mice housed under standard conditions.

The most consistent housing effects were found in the NMRI strain. During the whole 24 h period enriched housed mice spent more time on grooming and drinking, whereas standard housed animals spent more time on climbing and sleeping. In the BALB/c strain evident housing effects were found 13-18 h after the start of the experiment when the lights turned on again. However, the differences were not consistent with the rest of the period, e.g. enriched housed animals showed more climbing, but in the period from 1-13 h, standard housed animals showed more climbing.

In both strains enriched housed mice spent more time on eating (but only significant for the BALB/c mice). This finding seems to differ from previous studies (Van de Weerd et al 1994; Van de Weerd et al submitted/b), in which enriched housed mice were found to eat less than standard housed mice. The LABORAS system, however, measures eating behaviour (eating from the food hopper and eating a food particle clasped between the paws), in contrast with other studies which measure the amount of food which disappears from the food hopper. Similar observations apply for drinking behaviour.

In both strains climbing behaviour was found to be higher in the standard housed animals than in the enriched housed animals. These differences are mainly present in the first time period, but practically nil in the last time period. This is somewhat surprising, since the enriched housed mice have a climbing object in their cage (attached to the food hopper). Thus, higher climbing frequencies were expected in these groups, but this was only the case in the 13-18 h period of BALB/c mice. This suggests that standard housed animals were more active than enriched housed mice. Climbing is a part of exploration but excessive explorative climbing may lead to the development of stereotypies as was shown in ICR mice (Würbel et al 1996). Prior to the experiment the mice were housed in groups, but during the experiment they were individually housed. Isolation may have effects on the behaviour and physiology of an animal (Brain 1975). Mice in enriched environments may experience this isolation differently from mice in standard cages, which may be expressed in their behaviour. An indication that this could be the case are the lower levels of climbing in the enriched housed animals as compared with standard housed animals. A closer analysis of the first hours of the experiment, when the mice are exploring their cages may yield more information.

Significant differences between the strains were found. NMRI were less active (more grooming and sleeping), than BALB/c mice (more locomotion and climbing). The mice were housed in familiar environments, except that the cages were clean and they were housed individually during the test. It seemed that NMRI adapted faster to these new conditions by spending more time grooming and sleeping than the BALB/c mice, which showed more explorative behaviours (locomotion and climbing). BALB/c mice are known to be emotional and neophobic, they fear novel environments (Griebel et al 1993; Thiessen 1961), whereas NMRI mice are more tranquil. These different strain characteristics seem to be reflected in the behaviour of the mice.

The reactions of both strains to the housing conditions differed, for example, BALB/c mice from standard housing conditions showed more locomotion and drinking, whereas in the NMRI strain this was seen in the animals from the enriched conditions. Genetic differences between strains of mice may

influence the effects of different housing conditions as was shown for inbred mice in an earlier study by Van de Weerd et al (1994). Henderson (1970b, 1976) found that mice from enriched environments performed better on a food seeking task, but the size of the effect differed per strain and cross, suggesting genetic variability. Enrichment apparently interacts with the genotype of the animals used. This should be kept in mind when studying these effects.

A general problem in enrichment studies, is the fact that animals in enriched housing conditions are able to perform a wider range of behaviour than animals in standard environments and these different time budgets are difficult to compare (Novak et al 1995). The LABORAS system only registrates the pre-defined behavioural categories. Other (unknown) behaviours will be put into the undefined category. Behaviours such as for example nest building, which can be performed by the enriched housed mice because they have nesting material will thus be categorised as undefined behaviour. However, when comparing the levels of behaviour in the category 'undefined' for the two housing conditions, levels for the enriched housed animals were not higher. But since also other, for the system unknown behaviours are registrated in this category (e.g. digging), overall effects may remain undetected. In future, the system will be improved in order to registrate a more refined ethogram.

In conclusion, effects of environmental enrichment on the circadian rhythms of mice were not detected. However, consistent behavioural differences between the two strains of mice and differences in time budgets of mice housed in two different environments were found. The LABORAS system seems promising for future behaviour research.

### **Acknowledgements**

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