Final thesis

Behavioural effects of two different types of environmental enrichment on laboratory mice and rats.

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Abstract
The aim of this thesis was to investigate impact of a nesting material enrichment for mice and foraging enrichment for rats and how that would affect the animals, with emphasis on anxiety, exploration behaviours and long term effect. In experiment I, female mice of the strain NMRI were placed in either enriched housing system containing nesting material, or a control cages. Mice behaviour in the home cage was observed during two periods with one week in between to determine if the enrichment was still being used. Risk assessment and anxiety behaviours were observed in an open field test. During the second period the mice did climb and dig more while nesting, grooming, feeding and sleeping were performed more in the first period. Nesting was performed significantly more in the enriched cages, 18.63 % of the observations. The results obtained from the open field test were contradictive since mice in enriched housing showed more explorative *and* anxious related behaviours.
During experiment II, enrichment in the shape of a maze, was introduced to male rats of the strain Sprague-Dawley. The observation procedure was performed in the same way as in experiment I. Food was placed in the corners of the maze for the rats to hoard or to eat at the spot, the control cages was provided food ad libitum. On average the rats did spend 75.38 ± 3.31 percentage of the observation in the maze and 52.30 ± 19.29 in the cage. In the open field test the rats without enrichment showed more anxiety related behaviours. Enrichment may increase the ability to perform species specific behaviours, hence enhance welfare.

Keywords: Animal welfare, foraging enrichment, housing conditions, nesting material, stereotypies

1. Introduction
Environmental enrichment (EE) research dates back to the early 1980´s, with the goal of improving animal welfare by modifying housing condition (Olsson *et al*., 2003). According to Newberry (1995) EE is an improvement of the biological functioning of captive animals resulting from modifications to their environment. Thus enriched housing conditions allow animals to display a more extensive repertoire of species specific behaviours and may provide appropriate stimulation to facilitate coping with physical, as well as ethological needs (Van de Weerd *et al*., 1997, Van der Harst *et al.*, 2002). Experiments performed by Marashi *et al*., (2004) demonstrate that animals, provided with enrichment such as rats and mice, have increased their locomotory / exploratory activity, learning ability, problem solving behaviour, and decrease anxiety.

Commonly environmental design for laboratory animals addresses ergonomic and economic needs rather than animal welfare needs, as illustrated in several rodent species (Van de Weerd *et al.*, 1997, Sherwin & Glen 2002, Augustsson 2004, Baumans 2004). In order to reduce data variation and improve reproducibility of research results, most strains of rats and mice are inbred to such an extent that all individuals are in principle genetically identical. Further, the animal's environment is kept simplistic to controlling variability and thus including only such features that are essential for maintaining physical health and reproduction (Van Zutphen *et al.* cited in Olsson *et al.*, 2003). Laboratory housing under those conditions can deprive animals of the possibility to perform species-specific behaviour and may thus cause abnormal behaviours or stereotypys (Jensen 1996).

The animals used in these experiments are probably those most frequently used in laboratories around the world, *Mus musculus* and *Rattus norvegicus* (Augustsson 2004, Baumans 2004, Kaliste & Mering 2004). These species are typically kept in rather barren environments with limited possibility to perform species specific behaviours, where housing conditions are dictated by the concerns for human welfare rather than the animals (Jensen 2002). Mice and rats are nocturnal animals that live in social groups with dominant males and reproductive females. Rats and mice in the wild have several behavioural needs which include resting, nest building, hiding, and exploring, foraging, gnawing and social contact. (Olsson *et al.*, 2003, Baumans 2004, Kaliste & Mering 2004). When creating housing systems for laboratory animals, as many of their natural behaviours as possible should be taken into account. A behaviour that can occur if performances of natural behaviours are inhibited is barbering; whiskers trimming and sometimes body hair plucking from them or a cage mate. This is a rather common behaviour, assumed to be dominance behaviour and therefore overlooked as the welfare problem it might be, as Garner *et al* 2004 found that it was not dominance behaviour. They have seen that even singled housed mice have a tendency to barber themselves. According to Van den Broek et al 1993, (cited in Sarna *et al*., 2000); barbering can be a form of coping with inappropriate housing environment thus an indicator of poor welfare and abnormal behaviours.

Nesting material as an example is a strongly preferred enrichment for laboratory mice (Van de Weerd *et al*., 1997, 1998, Van Loo *et al*., 2002) given the fact that it allows them to structure their cage, somewhat control the environment, seek shelter and perform species specific behaviour to some extend (Van Loo *et al*., 2002, Baumans 2004). Except for the supposedly need of perform nesting behaviours, a behaviour prevalent in rats is food hoarding for future retrieval (Charron 2004., Day *et al*., 2003, 1999.,Dringenberg *et al*.,1998 Ellison 1995). This behaviour is commonly

seen foremost in male rats in laboratory conditions (Wallace 2003). Foraging rodents, like rats, are always under the risk of being detected by predators when exposing themselves outside the nest, thus food-carrying has an advantage by shortening the time outside the nest, especially larger pieces of food that will take longer to eat (Onuki & Makino 2005). Therefore we expect to see some food hoarding behaviour preformed by the rats.

A way of testing long-term effects of enrichment is to use an open field test. Given that when mice and rats face a new environment, they tend to search for potential threats, exploring the area for resources and to get familiar with the environment (Augustsson 2004), a method to quantify and analyse rodent anxiety and risk assessment behaviour is to use the open field test, which analyses activity and anxiety profiles (Scmitt and Hiemke 1997). During this test animals are place individually in an empty arena and observed to assess how explorative activity and emotionality are performed; which are measured by the animals risk assessment behaviours (Lebo 1953, Augustsson 2004). Positive thigmotaxis, or wall-seeking is the tendency to stay close to vertical structures such as walls, and this is something mice, together with avoiding open spaces, have a tendency to do (Choleris *et al*., 2001 cited in Augustsson 2004, Ohl 2003). Exploratory behaviours in rats and mice include sniffing, rearing, walking, climbing, manipulating objects and risk assessment behaviours. Due to that exploration is gradually inhibited by anxiety, hence its represents an indirect measurement of anxiety (Ohl 2003).Given that risk assessment is thought to be an active defence pattern, thus being closely related to anxiety (Ohl 2003), the risk assessments behaviours and anxiety behaviours can be described as gaining information about a novel situation and a means to determine if an actual threat is present (Augustsson 2004). According to Pinel & Mana 1989 risk assessment is also part of the cognitive decision making process, hence cost vs. analysis. Examples of risk assessment behaviour are: *Rearing*: standing up on its hind legs or leaning against the wall with its front paws, sniffing in mid air.*Grooming*, licking or cleaning themselves can be seen both as a displacement behaviour or body care, as displacement behaviour due to anxiety if the length of the behaviour is short, as body care if the behaviour is fully performed from head to tail (Spruijt *et al*., 1992). *Sniffing* in mid air, sitting or standing with obvious whiskers movement is considered as an exploratory behaviour. *Freezing*: Sitting on the ground or standing in a position with all paws on the ground, no obvious movement or sniffing, seen as anxiety behaviour. *Stretched attended posture* is when the mouse elongates its body posture and performing intention movements in different direction with its front paws, rear paws still. *Number of fecal boli* also

indicates anxious behaviour (Hall 1934 cited in Lebo 1953, Carola *et al*., 2002, Augustsson 2004).

The aim of this thesis was to investigate impact of a nesting material enrichment for mice and foraging enrichment for rats and how that would affect the animals, with emphasis on anxiety and exploration behaviours and if they will engage in the enrichment and the long term behaviour effect of it. Based on previous work it was assumed that well designed housing systems and enrichment allows for effective coping behaviour and may enhance welfare (Wechsler 1995., Van de Weerd *et al*., 1997) Therefore we would expect to see more explorative behaviours in an Open field test, less stereotypies/ abnormal behaviours in their home cages, and less stress related behaviours in the animals in the enriched housing system. The rats were expected to carry to eat or carry to leave the food pellets from the food source to the nest box. Results were obtained from behavioural studies in their cages and in open field tests.

2. Materials and methods

2.1 Experiment I

In this experiment nesting material was introduced as enrichment for female mice and compared to control mice without enrichment. Assessment of behavioural differences was determined in both open field tests and general behaviours in their home cage.

2.1.1Animals and housing conditions

Given that male mice can show severe aggressive behaviour towards each other (Bronson, 1979, Van Loo et al., 2002, Olsson et al., 2003, Marashi et al., 2004) 30 female mice from Scanbur BK Sollentuna, of the outbred strain NMRI were used for this study.
The mice arrived, 5 weeks of age, and were randomly placed, three to a cage (Tecniplast Macrolon 3), in one of two housing condition, enriched or control. Since mice are nocturnal animals their diurnal cycle was adjusted so that dark hours occurred between 0900 and 1800.Dull red lights were used during the dark period and two bright lights were turned on to simulate daylight.

2.1.2 Enrichment

In this experiment the mice were provided with nesting material mixed *with* the sawdust (instead of receiving it as a secondary bedding material).To get the pieces of tissue approximately the same size as the sawdust (2x5mm) a flat-iron was used to press the tissues, after that they were manually cut and

mixed with sawdust, 10 g in each cage and after each cleaning day. (Fig 1).Water and food were provided ad libitum for both treatments.

Figure 1 .Nesting material

2.1.3 Observation and experimental procedure

Upon arrival the mice were habituated to their new environment for seven days. After habituation, observations began. Every second day the cages were cleaned and sawdust mixed with new nesting material was placed in the enriched cages. The mice were handled by the same person during the study. The animals were individually marked every day on their back with a purple colour (Gentiana). The first mouse was marked with one stripe across the lower back, the second with two stripes and the last one with none. The last mouse was still handled as if she would be marked every day.

During the first period observations were performed during 4 days in which mice behaviours (Table 1) and the time spent manipulating the enrichment were observed After 4 days a break in the observation was initiated, to determine if, after a week (period 2) the enrichment was still being used to the same extend.

1/0 sampling was used with a 20 second interval for each focal mouse, hence 1 min/cage. The observation and behaviour sampling continued for 1 hour and 4 hours a day. The first day observations was performed 10-16, and 14-20 the second day. The third day observation took place 14-20 and during 10-16 the last. After one week the same procedure was performed again.

*Table 1. Mouse ethogram. *Open field behaviours.*

Nesting	Unfolding, carrying or in another way manipulating the nesting material.
Grooming	Licking/cleaning themselves or a cage mate.
Climbing	Climbing in the roof or on the food hopper no feet on touching the ground.
Feeding	Manipulating food or eating/drinking, coprophagy included.
Mounting	Trying to mount/mate with a cage mate
Sleeping	Sleeping or resting, separated or together with cage mates, no obvious movements.
Other behaviours (OB)	Sitting on the ground or standing in a position with all paws on the ground, or walking in a none precise direction.
Fighting	Chase, bite or being chased by a cage mate
Digging	With its forepaws or hind paws shuffle sawdust in a way that is not digging for food.
Rearing*	Standing up on its hind legs or leaning against the wall with its front paws, sniffing in midair.
Grooming*	Licking or leaning themselves
Sniffing*	Sitting/ standing and sniffing with obvious whisker movements.
Freezing*	Sitting on the ground or standing in a position with all paws on the ground, no obvious movements or sniffing.
Stretched attended posture*	Elongating the body and performing intention movements in different directions with its front paws, rear paws still.

2.1.4 Data analysis

Because individuals within one cage could not be considered to be independent in the analysis, mean values per cage were calculated for each time period (1 and 2). Behavioural data were expressed as cage means ± SEM. To analyse differences in behaviour between the experimental groups I used repeated measures ANOVA (within-subject factors: time period; between-subject factor: enrichment). The behavioural differences between the groups in the open field test were analysed by a *t* test SPSS 11.5 was used for all statistical calculations.

2.1.5 Open field test

An empty arena (∅120cm with 25 cm high walls), in a separate room, served as the open field test containing three zones, the outer area zone 1, the middle zone 2 and the centre zone 3. The cage containing the three mice was removed from the observing room and placed in the test room. The mice were tested individually. The observation started when the mouse was placed on the floor near the wall in zone 1. The test continued for eight minutes and 1/0 sampling was used. After being tested, the mouse was returned to its home cage. The arena was then cleaned with alcohol with the fecal boli counted and removed. The enriched and non-enriched mice were tested on consecutive days. The behaviours observed were; grooming, sniffing, rearing, freezing, stretched attended posture and number of fecal boli (Tab 1). All mice were handled by the same individual.

2.2 Experiment II

During this experiment a foraging enrichment, in the shape of a maze, was introduced as enrichment for male rats and compared to a control cage without enrichment. Assessment of behavioural differences was determined in an open field tests and general behaviour in the home cage.

2.2.1 Animals and housing condition

I used 36 male rats from Scanbur BK Sollentuna of the strain Sprague-Dawley, and randomly placed them, 3 to a cage in 12 cages (Ehret Marsinsreol VTH 2317/5), of which 6 cages were control cages without enrichment. They were anaesthetized with Isoflurane and marked with ears clips (1005-1 Monel), the first rat in the right ear, the second in the left and third not at all, but still handled as if he was marked.

2.2.2 Enrichment treatment

The enriched cages had a second floor of Plexiglas constructed as a maze for exploration (Fig 2). In two corners of the maze, food pellets were placed in a limited amount. New food was provided every second day and if they had collected some in the nest box, it was removed preventing a decrease in food handling and collecting. They had nest boxes on the first floor enabling them to climb up to the maze. Sawdust was spread both in the maze and on the first floor, water was provided ad libitum on the ground floor. The control cages were provided with water and food ad libitum, and sawdust covered the floor, but no maze or nest box. (Fig 3)

Figure 2. Second floor (maze) constructed of Plexiglas.

Figure 3. Control cage without the enrichment maze.

2.2.3 Observation and experimental procedure

As in the experiment 1, the daily rhythm of the rats was changed and a red light was used, starting at 0900 and turned off at 2100. They were habituated for seven days in their new environment without access to the maze. After habitation, food was placed in each of the two corners of the maze.
Every second day new food pellets were provided in the corners of the maze and the pellets that had been stashed in the nest box were removed and counted in order to prevent overweight and decrease in collecting behaviours. The cages were not cleaned during the test.

The observation was performed during 4 days in which rat behaviours were observed as well as the time spent in the maze (Tab 2). After 4 days a

break in the observation is initiated, to see, a week later if the enrichment is still being used to the same extend.

1/0 sampling was used with a 20 second interval for each focal mouse, hence 1 min/cage. The observation and behaviour sampling continued for 1 hour and 4 hours a day. The first day observations was performed 10-16, and 14-20 the second day. The third day observation took place 14-20 and during 10-16 the last. After one week the same procedure was performed again.

*Table 2. Rat ethogram. *Open field behaviours*

Grooming/maze	Licking/cleaning themselves or a cage mate.
Feeding	Manipulating food or eating/drinking, coprophagy included.
Feeding maze	Carrying food in the maze or eating at the spot.
Mounting/maze	Trying to mount/mate with a cage mate.
Sleeping/maze	Sleeping or resting, separated or together with cage mates, no obvious movements.
Other behaviours (OB)	Sitting on the ground or standing in a position with all paws on the ground, or walking in a none precise direction.
Fighting/maze	Chase, bite or being chased by a cage mate.
Digging	With its forepaws or hind paws shuffle sawdust in a way that is not digging for food.
Digging maze	Scratching/gnawing or digging in/on the maze.
Rearing	On its hind legs standing and sniffing in midair, leaning against something or not.
Out of sight	Situated in a way not visible for the observer.
Rearing*	Standing up on its hind legs or leaning against the wall with its front paws, sniffing in midair.
Grooming*	Licking or leaning themselves
Sniffing*	Sitting/ standing and sniffing with obvious whisker movements.
Freezing*	Sitting on the ground or standing in a position with all paws on the ground, no obvious movements or sniffing.

2.2.4 Data analysis

Because individuals within one cage could not be considered to be independent in the analysis, mean values per cage was calculated for each time period. Behavioural data were expressed as cage means ± SEM. To analyse differences in behaviour between the experimental groups I used repeated measures ANOVA (within-subject factors: time period; between-subject factor: enrichment). Differences in behaviour between the groups in the open field test were analysed by a *t* test.
SPSS 11.5 was used for all statistical calculations.

2.2.5 Open field test

An empty arena (∅120cm with 25 cm high walls) served as the open field test containing three zones; the outer area zone 1, the middle zone 2 and the centre zone 3. The rats were tested individually. The observations initiated when the rat was placed on the floor near the wall in zone 1. The test continued for four minutes and 1/0 sampling was used. After being tested, the rats were returned to its home cage. The arena was then cleaned with the fecal boli counted and removed. The enriched and non-enriched rats were tested on consecutive days. The behaviours observed were; grooming, sniffing, rearing, freezing and number of fecal boli (Table 2). All rats were handled by the same person.

3. Results

3.1 Experiment I

3.1.1 Undisturbed behaviour in home cage

As seen in Table 3 the enrichment treatment only showed significant changes in the nesting behaviour.The animals always used the material for nest building, starting soon after new material was provided.
Significant differences in all the behaviours in the time aspect (period 1 and period 2), except mounting, OB, and fighting behaviours. Nesting, grooming, sleeping and feeding were performed significantly more in the

first period of the experiment while climbing and digging were observed more in the second period, 61% and 68% more respectively (fig 4a-d).

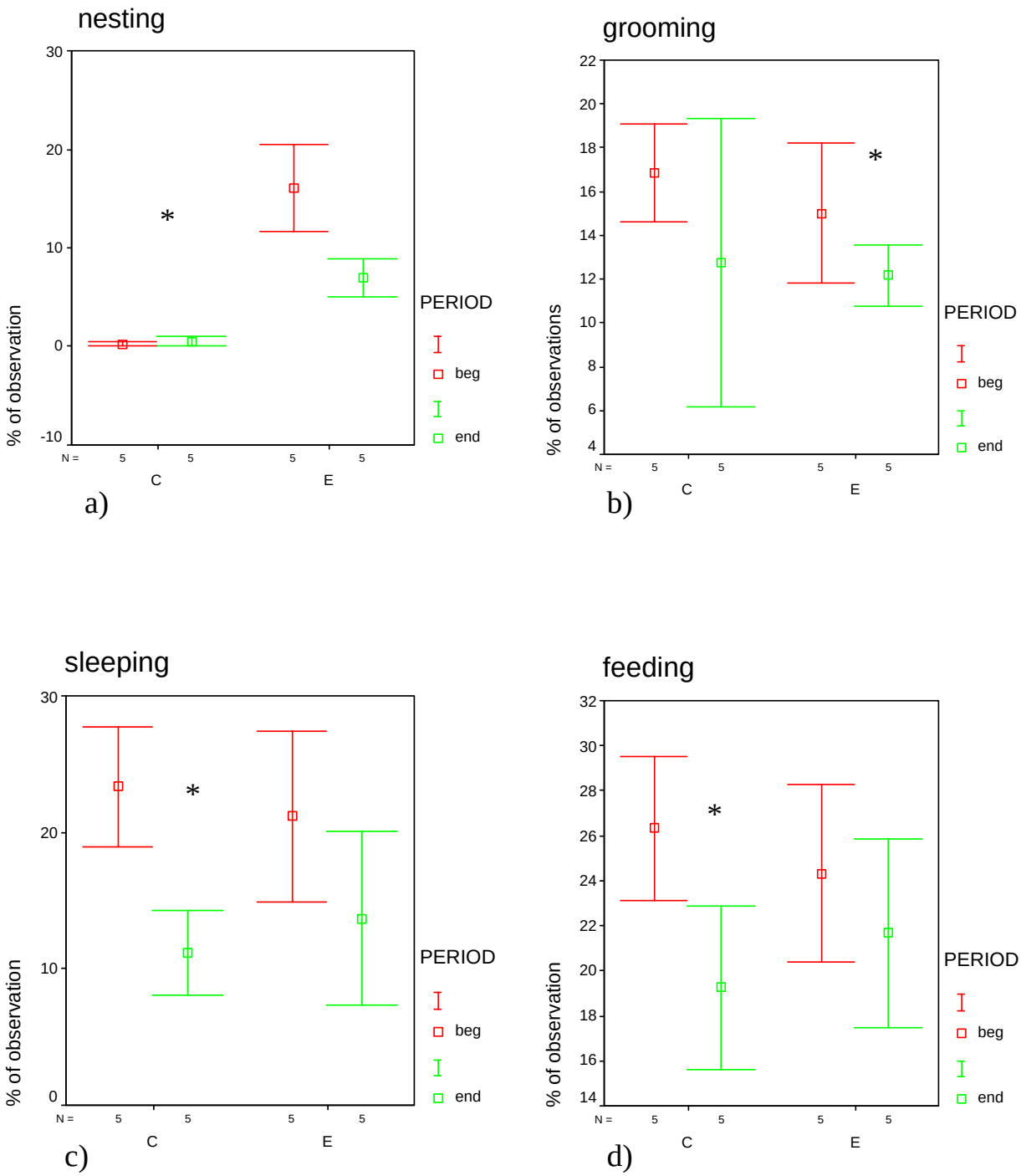


Figure 4. Percentage of obseravtions in which the mice were a) nesting, b) grooming, c) sleeping, d) feeding. Where c= control housing and E = enriched housing.

Two mice in the same cage from the non enriched housing had barbered whiskers and two more showed signs of being barbered (fig 5). The barbered mice also showed tendencies of stereotypys; performing the same climbing ritual in the bars of the roof and extensive digging behaviour with no apparent consequence or reason.

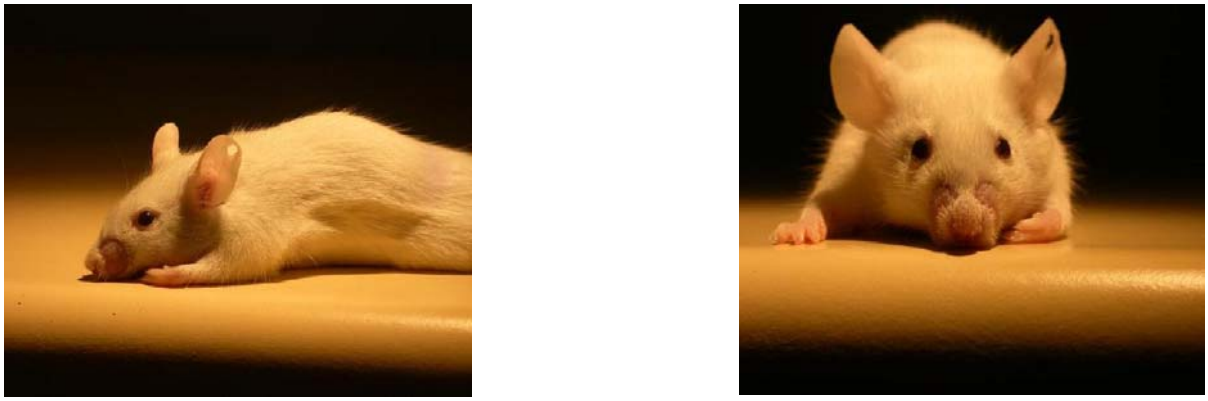


Figure 5. Barbered mice from the control housing system.

Table 3. Results obtained from the ANOVA test. Significance was seen in nesting, grooming, climbing, sleeping and feeding for both the time and enrichment aspect.

Behaviour	Time			Enrichment		
	Df	F	p	Df	F	p
Nesting	2	17.375	.003	2	69.524	<.001
Grooming	2	5.077	.054	2	.281	.611
Climbing	2	134.08	<.001	2	1.092	.327
Mounting	2	.325	.584	2	1.923	.203
Sleeping	2	31.098	.001	2	.003	.960
OB	2	4.466	.068	2	.080	.784
Fighting	2	7.806	.231	2	2.695	.139
Feeding	2	18.276	.003	2	.008	.993
Digging	2	8.522	.019	2	1.693	.229

3.1.2 The open field test

Movement from zone 2 to zone 3 ($p < 0.019$), zone 3 to zone 2 ($p < 0.09$) and the stretched attended posture ($p < 0.02$) were all performed significantly more by the enriched mice in the open field test arena, during the 8 minutes of observation period. (Fig 6a-c)

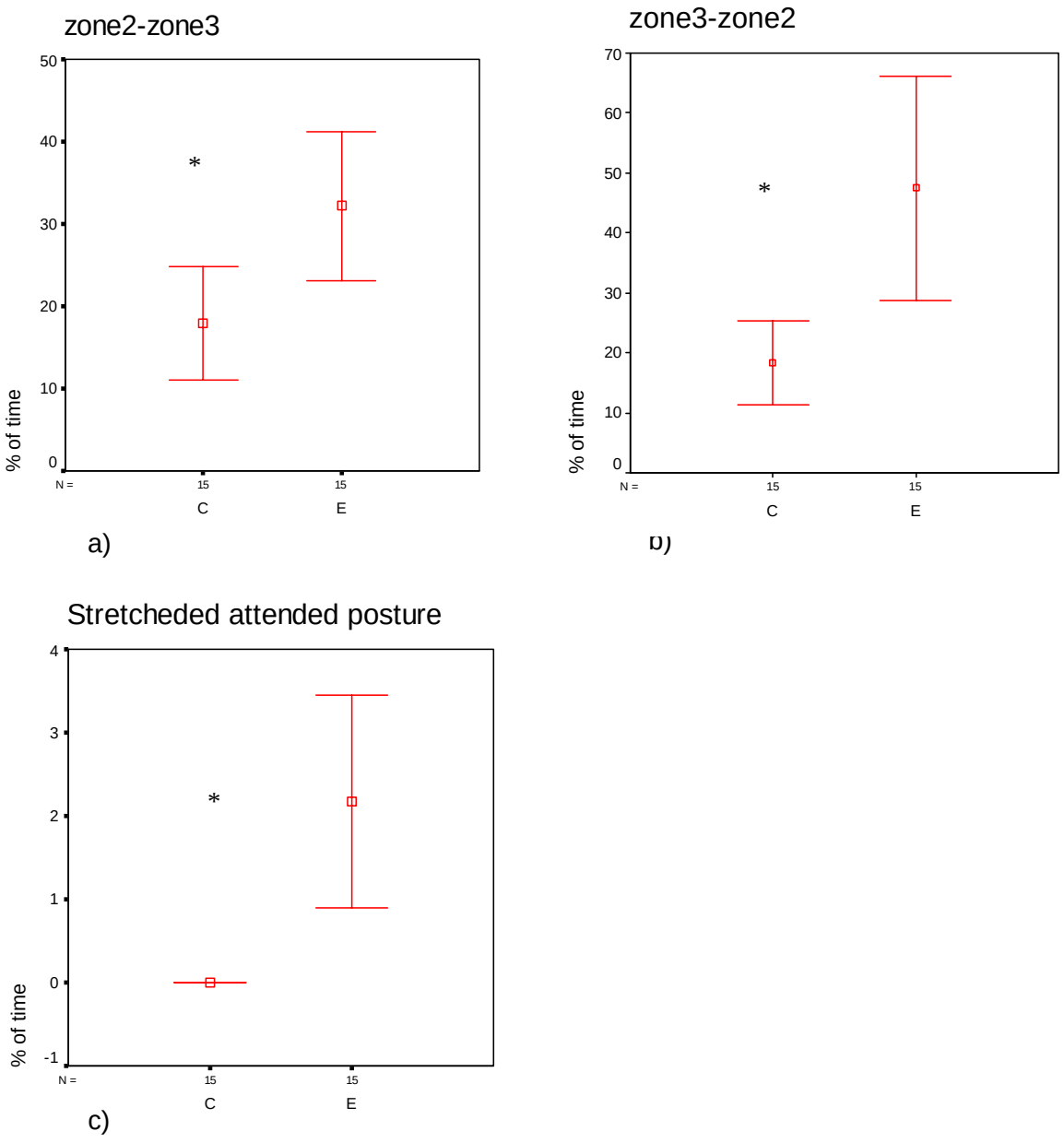


Figure 6. Percentage of observation in which the mice were. a) Walking from zone2- zone3, ($p < 0.019$), b) walking from Zone 3 - zone 2 ($p < 0.09$), c) showing stretched attended posture ($p < 0.02$). Where C= non-enriched housing and E= enriched housing.

3.2 Experiment II

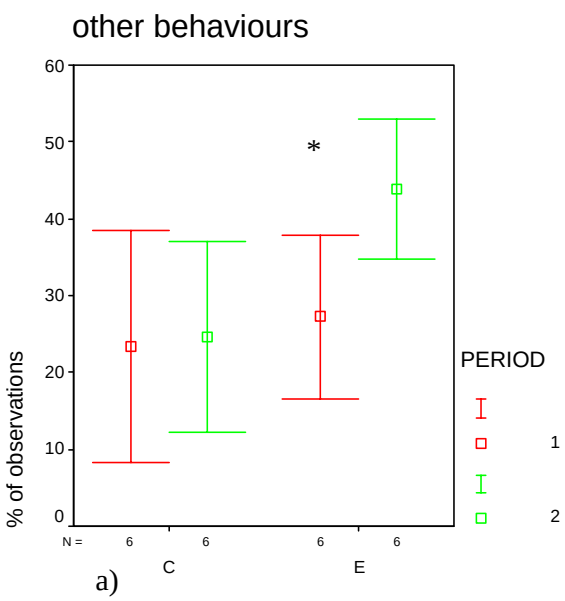
3.2.1 Undisturbed behaviour in home cage

There were significant differences between period 1 and period 2 in regards to OB, feeding, rearing and in the out of sight behaviours between the groups of rats. The rats in the enriched cages performed more other behaviours (OB), ate more and slept more the second period of the study. The rats in enriched housing further differed from the control groups in the time being out of sight which occurred more in the first period (Fig 7a-g).

The enrichment aspect of the study showed significant difference from the control rats in all behaviours except fighting, OB and the feeding behaviours. The grooming, sleeping and rearing behaviours were performed more by the rats in control cages whereas digging, and out of sight were performed to a greater degree by the rats in enriched cages (Table 4). On average the rats did spend 75.38 ± 3.31 percentage of the observation in the maze and 52.30 ± 19.29 in the cage (Fig 8.). No food hoarding was seen throughout the experiment.

Table. 4 Results from the ANOVA test. Significance was seen in other behaviours (OB), feeding, rearing, out of sight, grooming, digging and out of sight.

Behaviour	Time			Enrichment		
	Df	F	p	Df	F	p
Grooming	2	.05	.828	2	5.510	.041
Digging	2	2.71	.131	2	8.380	.016
Fighting	2	.037	.851	2	2.729	.130
Sleeping	2	.891	.367	2	7.192	.023
OB	2	8.916	.014	2	2.092	.179
Feeding	2	4.894	.051	2	1.383	.267
Rearing	2	17.893	.002	2	81.495	<.001
Out of sight	2	9.468	.012	2	20.589	.001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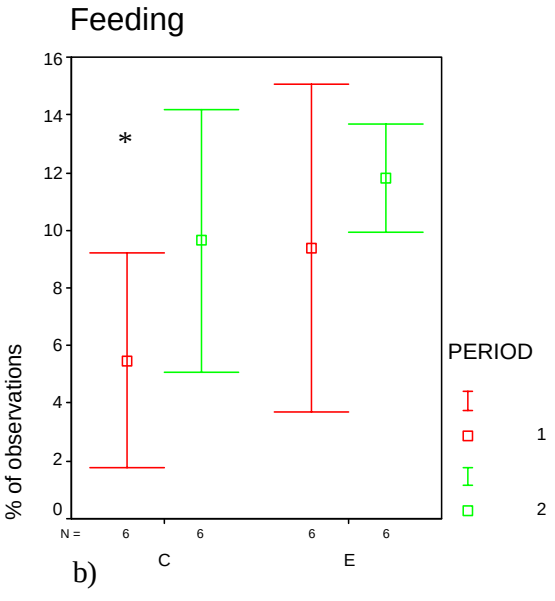
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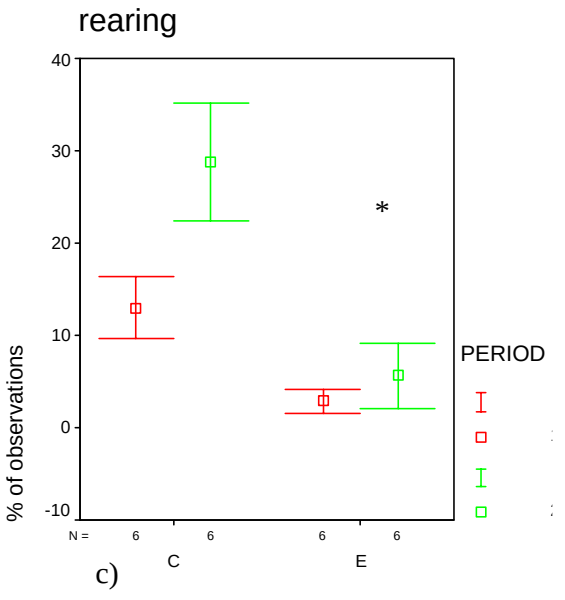
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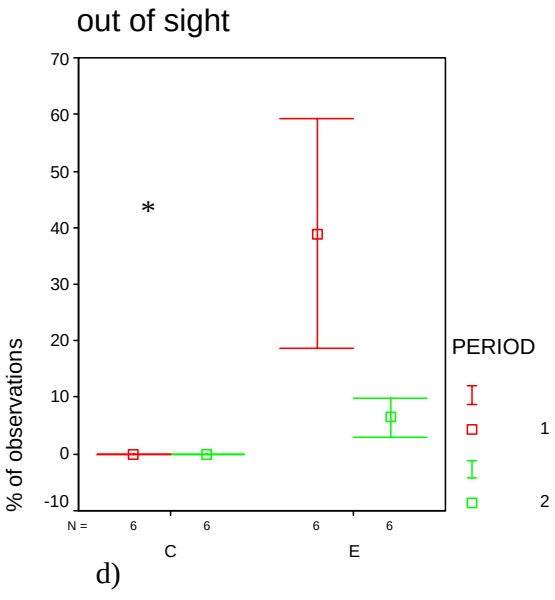
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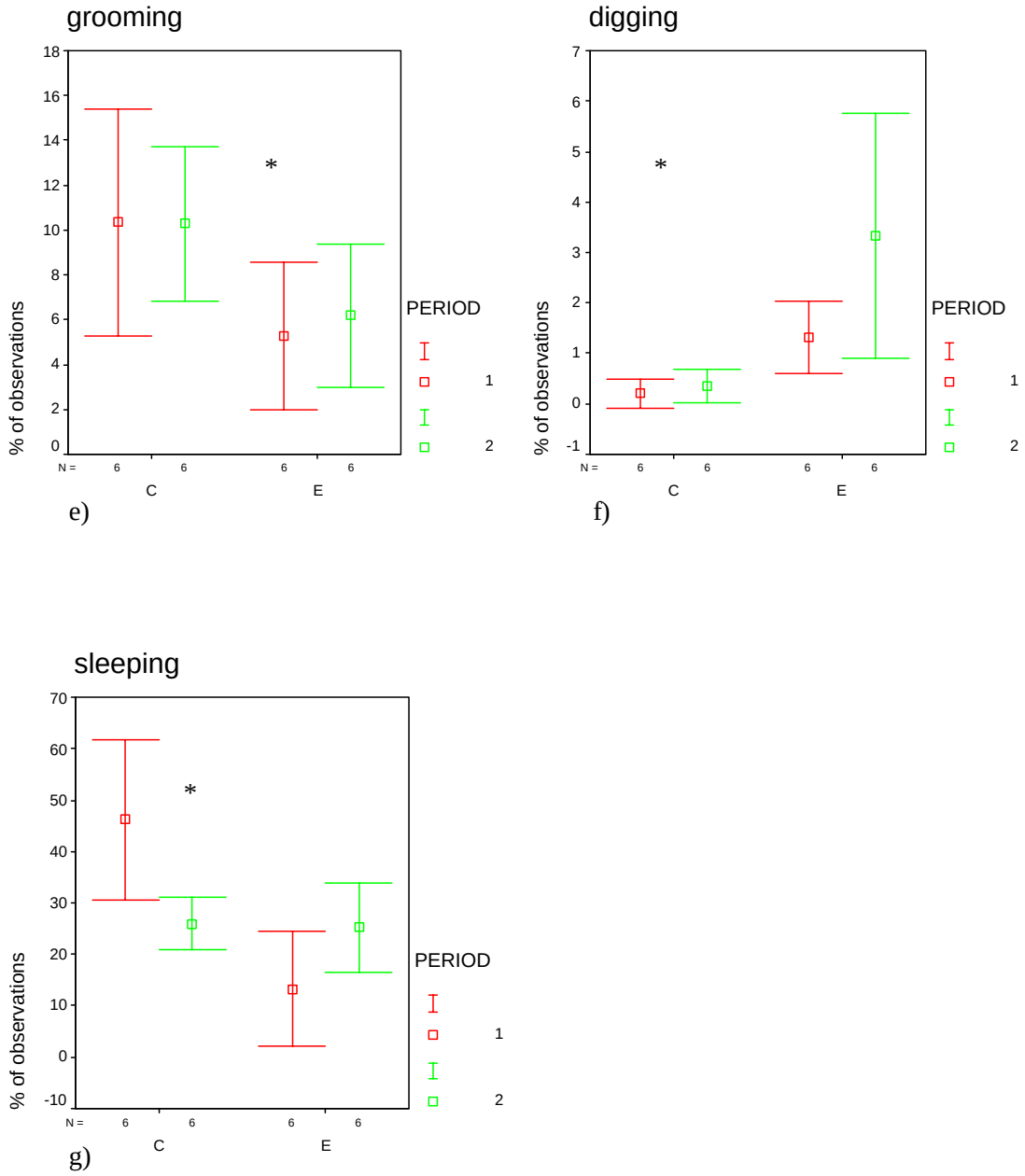

Figure 7. Percent of behaviours in which the rats were a) performing other behaviours, b) feeding, c) rearing, d) are out of sight, e) grooming, f) digging, g) sleeping. Where c= control housing and E= enriched housing.

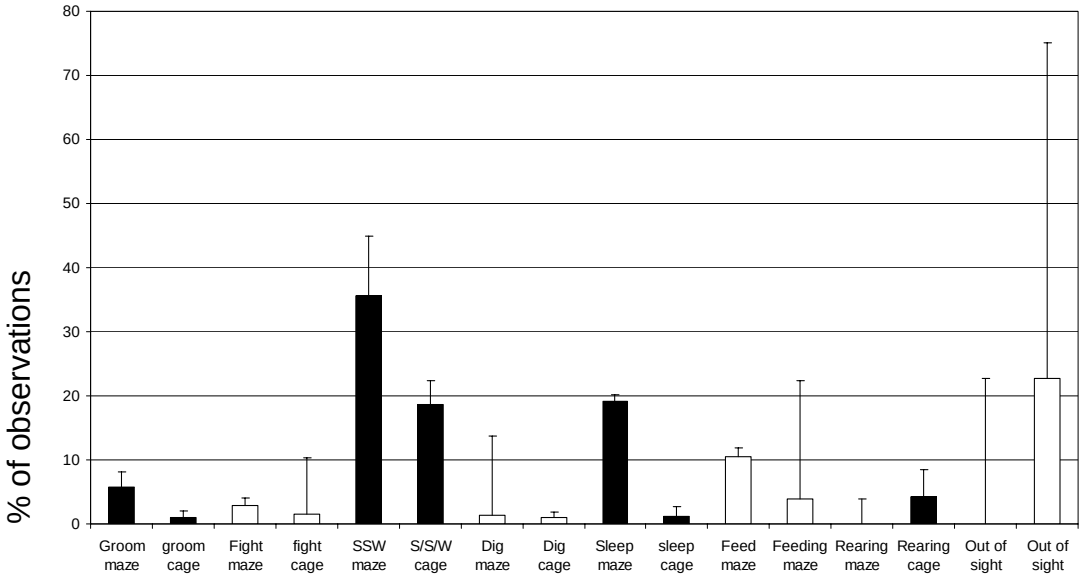

Figure 8. Average % of observation in which the rats in enriched cages performed different behaviours in the maze or in the cage

3.2.2 The open field test

Rearing ($p < 0.022$) and walking from zone 1 to zone 2 ($p < 0.037$) and from zone 2 to zone 1 ($p < 0.042$) were all performed significantly more by the rats without enrichment in the open field test arena, during the 4 minute observation. (Fig 9a-c)

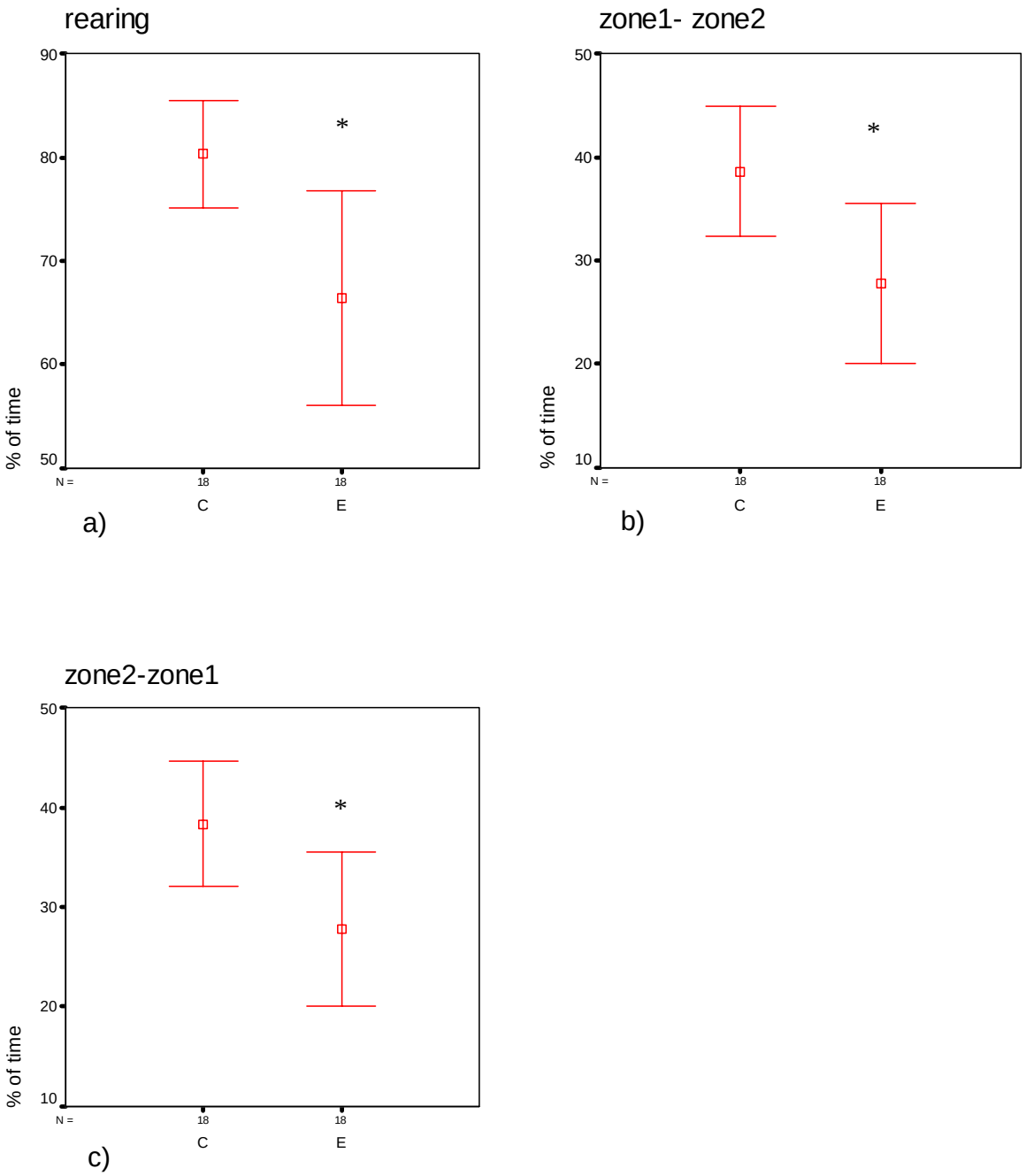

Figure 9. Percentage of observation in which the rats were. a) Rearing ($p < 0.022$), b) walking from Zone 1 - zone 2 ($p < 0.037$), c) walking from zone 2- zone1 ($p < 0.042$). Where C= non-enriched housing and E= enriched housing.

4. Discussion

A simple form of enrichment in the standard cage for laboratory mice is providing them with nesting material, which reduces the occurrence of stereotypic behaviours and barbering as shown in this study. Assumed stereotypic behaviours like climbing and digging were performed 61% respectively 68% more in the second period and no stereotypies at all observed in the enriched mice. Enriched cages have been shown to be rewarding to rats providing the animals with the ability to show a more extensive repertoire of behaviour (Van der Harst *et al*., 2003). Subdividing the cage into two compartments, a nest box and a maze also allows the rat to avoid conflicts with cage mates, thus decreasing the frequency of aggressive behaviour (Van der Harst *et al*., 2003).

4.1 Experiment I

Tissue paper (Kleneex) has been show to be highly appreciated as nesting material (Van de Weerd *et al*., 1997, 1998) because it gives the mice an opportunity to perform species specific behaviours and the potential for manipulation. This possibility to structure the environment and building nest may reduce boredom and stress by providing a hiding place.

There was no significant difference between the two housing systems considering the enrichment aspect except the nesting behaviour, which was performed 18.63% of the observations. However, if we look to the time aspect (period 1-2) one could see significant difference in grooming, climbing, sleeping, feeding and digging, for both treatments There was a clear difference with climbing and digging performed significantly more during the second period. According to Würbel *et al*., (1998) stereotypies start to show after 24 days, after 34 days an early stage of stereotypy is seen and it is fully developed after 80 days. In this study the mice were approximately 50 days old when the second period started. This could explain why there were more observations of climbing and digging as seen in Würbel *et al*., (1998) who observed that stereotypies developed in gerbils that were prevented from retreating into burrows in standard housing systems. Since the enriched mice lacked stereotypies one could draw the conclusion that manipulating the nesting material inhibited development of stereotypies. Barbering, whether dominance or abnormal behaviour but not a stereotypy, according to Garner *et al*., (2004), was present in some of the mice in this study. Even though earlier papers (e.g. Long 1972) suggest that barbering must be an act of dominance Garner *et al*., (2004) dispute this statement. They found that the severity of hair loss seen in barbered mice was related to the relative dominance between the barbered mice, but not to the barber. This study may demonstrate that barbering is an act derived from boredom and best described as an

abnormal behaviour, since it was only present in the mice in the control housing system. This statement is supported by a study by Garner *et al*., (2004) which found that providing mice with nest building behaviour helps control the behaviour. The mice in this study were not housed for more than a couple of weeks which could be the reason barbering did not developed in more of the control cages since possible stereotypies didnt have the time to fully develop.

Würbel *et al*., (1998) found that time spent eating decrease with age thus animal age could explain the significant difference in time spent feeding between period 1 and 2 in this experiment. Time spent eating was the same in the two housing, a finding that differs from previously reported findings (Van de Weerd *et al*., 1997).

The predominant and most consistent difference, except the ones in the time aspect, between mice from enriched and standard housing conditions was seen in the open field test. Here the enriched mice showed higher locomotion level from zone2-zone3 and back. These results, interpreted as higher exploratory behaviours, are in concordance with Van Roy *et al*., (2001) who also scored higher frequencies of locomotion in an open field test with mice under enrichment conditions. However they observed fewer stretched attended postures in their experiment and the mice in this study showed more stretched attended postures than the control animals. Stretched attended posture is seen as risk assessment behaviour, described in Augustsson (2004) as “gaining information about a novel situation and a means to determine if an actual threat is present”. In this study the enriched mice had access to nesting material, thus shelter. When subjected to a novel environment, such as the open field arena, without shelter the enriched mice may have experienced a greater amount of anxiety than the mice without enrichment, that lacked shelter, hence more stretched attended posture in the enriched mice.

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4.2 Experiment II

In this study rats clearly shows a preference to spend time in the maze rather down in the cage. Even though they did spend time in the maze no food hoarding was seen throughout the experiment even though the pellet size was about 2g. Dringenberg *et al*., (1998) has seen that pellet size has a strong influence on food hoarding behaviour in rats where small pellets were picked up with the mouth and eaten immediately whereas larger pellets <1g was carried to a nest box for consumption later (Whishaw *et al*., 1995, Onuki & Makino 2005).

The fact that the rats did not stash away the food is contradictory to the work of other authors but given that the maze was rather narrow and dark, this may explain the results, because the predator risk was not that striking. They carried the pellets, however, away from the food source and ate them as far away from the observer as possible. Day *et al*., (1999) previously demonstrated that when Siberian hamsters were fasted increased their food hoarding behaviour. With this in mind the rats in this study might have felt no need to hoard since they were given a large amount of food every second day. This speculation is supported by Day *et al*., (1999) which stated that food hoarding is negligible when presented ad libitum.

The time aspect of the study (period 1 and period 2) showed that feeding and rearing were performed more often the second period by both treatments and although OB also was performed more the second period it was only by the enriched treatment. Van de Weerd *et al*., (1997) and Würbel *et al*., (1998) found that feeding decreased with age and that contradicts the results obtained in this study where the rats ate significantly more the second period. The reason that the enriched rats were out of sight more during the first period may be due to a very anxious state in the beginning of the study. However after a few weeks they learned to use the maze and did spend more time there the second period as shown in the increase of locomotor behaviours the second period.

Between the enriched housing system and the control cage a difference was observed in grooming and sleeping where these behaviours was performed more by the control group. These two results contradict each other when grooming is said to show *less* in the animals with enrichment while sleeping is more prominent in an enriched system (Würbel *et al*., 1998). The fact that rearing occurred more in the control housing can easily is explained by the structure of the cage. In the control housing the roof was transparent and therefore inviting to rear, while in the enriched cage the roof was a maze through which the rats couldn't see. During the open field test the control rats reared significantly more and walked more from zone 1 to zone 2 and back. The zone walking can indicate that they felt more comfortable and secure near the walls of the test (positive thigmotaxis). Rearing is seen as anxiety behaviour, the result from the test is quite passable but on the other hand there was no significant difference in the locomotion or explorative behaviours.

4.3 General discussion

When scientific research involves animal use one should take into consideration their behaviours are factors that are all a product from the environment and genetics of the animal. Sherwin *et al*., (2004) suggest that the performance of behaviour per se is reinforcing to the animals, rather than the functional consequences. Researcher fear that enriched housing for

laboratory animals show more variability in their response to experimental procedures because they show more diverse behaviour, but the use of healthy animals is an important factor when performing scientific research (Augustsson 2004) and if the welfare of the animal is good, the research results should be more valid.
Van de Weerd *et al*., 1997 has shown that enrichment, even though important for enhancing the animals' welfare, did not alter scientific results or the physiology of the mice. Therefore no good reason seems to be found for depriving laboratory mice from nesting enrichment

5. Conclusion

There can be said that during this study mice provided with nesting material spend 18.63% of the time observed nesting, had less tendency to develop stereotypies and did not barber each other as predicted. The tendency for more exploratory behaviours in an open field test was also seen. Respectively the enriched rats did spend an average of 75.38 ± 3.31 percent of the observations in the maze, even if they didn't engage in hoarding behaviour as expected they obviously used the enrichment to a large extend.
However the present study provide some evidence that even simple enrichment may have great potential in reducing stereotypic behaviours, increase exploratory behaviour and may thus enhance the well-being in laboratory animals.

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