UPTEC X 06 041
OKT 2006
ISSN 1401-2138

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Maternal and nest building behaviour in laboratory mice

Master's degree project

UPPSALA UNIVERSITET

Molecular Biotechnology Programme

Uppsala University School of Engineering

UPTEC X 06 041	**Date of issue 2006-10**

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Title (English)

Maternal and nest building behaviour in laboratory mice

Title (Swedish)

Abstract

Research involving laboratory animals, such as mice, are performed all over the world. The difference between the environment in a laboratory cage and out in the wild affects the mice' development and behaviour. To prevent abnormal behaviours and improve the wellbeing of laboratory animals, it is therefore important to enrich the laboratory housing and evaluate the animals' behaviours.

Keywords

Maternal behaviour, Nest building behaviour, environmental enrichment, laboratory mice

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Project name	Sponsors
Language **English**	Security
ISSN 1401-2138	Classification
Supplementary bibliographical information	Pages **31**

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Maternal and nest building behaviour in laboratory mice

Frida Andersson

Sammanfattning

Försöksdjur används ofta inom medicinsk forskning för att man ska kunna förstå normala och onormala beteenden hos både människor och djur. Redan i början av 1900-talet började man använda möss som försöksdjur inom cancerforskningen. Sedan dess har användandet stadigt ökat tack vare nya tekniker som bland annat gör det möjligt att manipulera djurens gener. Möss har förmågan att kunna anpassa sig väl till nya miljöer men behöver stimulans för att utvecklas normalt och må bra. Om försöksdjur inte mår bra kan detta påverka forskningen som baseras på djuren. Det är därför är det viktigt att mäta och utvärdera försöksdjurens beteenden för att öka välfärden samt förstå effekten på forskningsresultat av djurens beteende.

Förlust av nyfödda kullar är ett problem vid användandet av möss i laboratorier. Vad detta beror på är inte känt. Kan det vara så att en standardmiljö påverkar hur musmammorna beter sig mot sina ungar och kan detta i så fall förhindras genom att berika miljön med enkla medel (t ex med kartongrullar, papper och bitblock)? Möjligheten att kunna uttrycka normala beteenden såsom bobyggande, gnagande och klättrande kan kanske motverka att vissa onormala beteenden, som kannibalism och repetitiva beteenden, utvecklas. Att studera beteenden är inte lätt i mössens naturliga miljö men inte heller i ett laboratorium. Mössen väljer att bygga bo där det passar dem och det kan ibland försvåra filmning och analys av deras beteenden. Därför undersöktes möjligheten att med hjälp av beräkning försöka kunna förutspå var de kan tänkas bygga sina bon.

Examensarbete 20 p i Molekylär bioteknikprogrammet

Uppsala Universitet oktober 2006

UPTEC X 06 041

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1 Introduction

1.1 General background

Research based on animals is used in many areas for trying to understand normal and abnormal processes in humans and animals. From being held as fancy animals (Sluyter and Oortmerssen, 2000) or seen as a pest animal (Morton, 2002) mice were initially taken into research in the beginning of the 20th century for cancer research (Sluyter and Oortmerssen, 2000). Studies within biomedical research involving transgenic and knockout techniques have lead to an increased use of laboratory mice (Olsson *et al*., 2003). Additionally, the fact that mice become sexually mature at 5-12 weeks of age (Latham and Mason, 2004) and that they have a short reproduction cycle (reviewed Weber and Olsson, In press) make them suitable for research. Female mice have an oestrus cycle that lasts between four and six days, gestation varies from 18-21 days (e.g. Latham and Mason, 2004) and they can rear a litter of 4-9 pups every sixth week under favourable conditions (König and Markl, 1987). This can vary between individuals, strains, season, diet and the environment (Weber and Olsson, 2006a).

Most mice used in laboratories descend from *Mus musculus*, a species spread all over the planet except for tropical Africa (Latham and Mason, 2004). Even though mice easily adapt to new environments (Latham and Mason, 2004; Sluyter and Van Oortmerssen, 2000) the restricted environment in a laboratory cage is very different compared to the environment out in the wild (Latham and Mason, 2004; Olsson and Dahlborn, 2002). In natural conditions, mice move around walking, rapidly running, climbing or jumping. They are also capable swimmers and can easily balance along thin ropes and wires and squeeze through narrow cracks (Latham and Mason, 2004). Experiences early in the mouse's life affect the phenotype so the pup develops into a grown up individual best suited for the local environment (Latham and Mason, 2004). Not being able to carry out normal behaviour might affect the animal's mood and thereby interfere with experimental results as will be further outlined in the following.

1.2 Behaviour studies

Measuring and understanding behaviour, meaning actions and reactions of organisms (Martin and Bateson, 1993), is important when it comes to welfare of laboratory animals and also for understanding effects on research results (Olsson *et al.*, 2003). Questions have been raised whether behavioural research is a waste of time and that focus should be on understanding underlying processes instead. But understanding mechanisms underlying behaviour, like neurophysiological and biochemical processes, must be combined with the understanding of the behaviour itself to be satisfactory (Martin and Bateson, 1993). Thus knowing that a mouse has a mutation in the

vomeronasal organ (the organ that detects pheromones and triggers behaviours such as territorial defence and mating) would not be of any use unless behavioural studies had shown that male mice with such defect could not recognise the sex of a conspecific and failed to display male-male aggression (Loconto *et al.*, 2003).

It is possible to measure behaviour within populations down to one certain individual's movements (Martin and Bateson, 1993), depending on which question is of interest. Experiments performed with selectively bred and genetically manipulated animals placed in small cages might yield misleading results. When it comes to observing mice, it is most of the time difficult to perform such studies in the animal's natural environment. Mice may live underground in simple or complex burrows, in wall cavities or other protected sites where they can build a nest (Latham and Mason, 2004). To be taken into the laboratory, selectively bred, genetically manipulated and placed in small cages without any chance to escape attacks, might be a source of pathologic artefacts (Olsson *et al.*, 2003). Not only visible surgical procedures can cause harm to a laboratory animal (Martin and Bateson, 1993). Social deprivation (Martin and Bateson, 1993) and lack of stimulation (Olsson *et al.*, 2003) might be as damaging and affect an animal's behaviour.

Behaviour studies in animal facilities are often performed with protocols and apparel developed in each laboratory. Different genotypes may respond differently to the same environment and standardisation of tests and environments may counteract failures in replicates of experiments (Wahlsten, 2001). On the other hand, creating a standard housing condition might increase the risk for obtaining results specific to a particular situation, not necessary guaranteeing the validity of research results (Würbel, 2001). The term ‘Environmental enrichment’ describes attempts to improve housing conditions for laboratory mice by providing a more complex cage environment with resources that enable the performance of motivated behaviours such as nest building. Laboratory animals’ physical needs are often cared for giving them well-balanced diets and keeping them in climate controlled facilities with good hygienic conditions. Expression of many normal behaviours is on the other hand not possible in a standard laboratory cage and this leads to a potential welfare problem (Olsson and Dahlborn, 2001). Behavioural abnormalities are commonly seen in laboratory animals housed in small cages with only litter material, food and water (Olsson *et al.*, 2003). When laboratory mice attempt to escape from their cages they have developed different forms of abnormal repetitive behaviours, known as stereotypies (Würbel, 2001). It has been shown that deer mice provided with early environmental enrichment show lower levels of stereotypy than animals from barren cages. Whether a sensitive period for the prevention/ development of stereotypies exists is still to be investigated (Hadley *et al.*, 2006). Environmental enrichment does not only affect the development of abnormal behaviours but have a wide spectrum of effects on

mice' behaviour and neurobiology, including increased complexity of the central nervous system accompanied by improved performance in tests of learning and memory. Rearing animals in 'enriched' cages may have effects that last well into adult life (Olsson and Dahlborn, 2002). Cage enrichment is not equivalent with a perfect environment for laboratory animals. Firstly, the cage size and practical considerations in the animal facility set limits to what can be achieved in terms of adaptation of the environment. Secondly, care must be taken when stimulation of natural behaviours such as aggression and protection of territories in group-housed male mice occurs as a response to the enrichment (Olsson *et al.*, 2003).

1.3 Maternal behaviour

A common problem among laboratory animals is loss of newborn litters (Weber and Olsson, 2006a). Whether this is due to environmental factors or poor maternal care is still not clear. Maternal behaviour is regulated by stimulation from the environment, the offspring and neurological interactions. Different strains have shown differences in maternal care and mutant mice display disorders of maternal care (Brown *et al.*, 1999; Thomas and Palmiter, 1997). Recent results also indicate a role of the environment in that litter loss was found to be much greater in barren cages than in cages furnished with shelters and nesting material (Weber and Olsson, 2006b). Perturbations of the mother-infant relationship can lead to behavioural consequences that persist for the entire life of the mouse. Reduced adult social behaviour, i.e. changes in learning and memory performance and behaviour in stressful situations, have been linked with variations in maternal care (Branchi *et al.*, In press). Behaviours directed at the pups are considered as maternal care; nest building, feeding, caring for and protecting them after birth (König and Markl, 1986). Not only female but also male mice show parental behaviour (Weber and Olsson, 2006a).

1.4 Nest building behaviour

Introducing environmental enrichment, such as nesting material, to laboratory cages allows mice and other rodents to express their natural nest building behaviour. Both female and male mice show nest building behaviour and prepare a nest before parturition (Weber and Olsson, 2006a). Nest building, an important part of maternal behaviour, is influenced by environmental temperature, maternal experience and presence of pups. It is also related to the reproductive success of mice (Bond *et al.*, 2002). But nest building is not restricted to reproduction: all mice build nest that they sleep in (Olsson and Dahlborn, 2002). A nest can serve as a shelter; it is also a place for the mouse to avoid light and where the animal can regulate its temperature. (Van de Weerd *et al.*, 1998) Mice can have preferences for different types of nesting material (reviewed in Olsson and Dahlborn, 2002) and it is therefore of interest to evaluate if animals respond to different enrichments (Van de Weerd *et al.*, 1998) before research involving new materials is conducted.

2 Experiment 1: Maternal behaviour study

2.1 Aim

The aim of this experiment was to compare maternal behaviour between female mice reared in two different environments. Five females reared in a standard environment were compared with six females reared in furnished cages. It was also of interest to see if there were any differences between behaviours during night and day for the laboratory mice.

2.2 Materials & Methods

2.2.1 Animals and housing

The experiment took place in the Laboratory Animal Science group at the Institute for Molecular and Cell Biology (IBMC) in Porto, Portugal. The experiment started in October 2005 and this part of the study was conducted from February 2006.

Females from the inbred strain C57BL/6J born in either furnished (standard wire-topped Makrolon III cages (265 x 410mm, height 175mm) containing sawdust litter, 1 dl of bedding sawdust, one chew block, half Kleenex sheet, a translucent red PVC nest box (MouseHouse, Techniplast) and a cardboard nest box (Des Res., Lillico)) or barren cages (standard wire-topped Makrolon II cages (265 x 205mm, height 140mm) containing only sawdust litter) were weaned at 22 days of age. The different housing systems during the rearing are shown in Picture 1. Post-weaning environment was the same for all females: they were housed in standard wire-topped Makrolon II cages containing sawdust litter, one cardboard tube (100mm long, 45mm diameter) and one sheet (2,3g) of absorbent paper (Renova SA, Torres Novas, Portugal)) in groups of 2-6 females (litter mates or weaned at the same time). Food (Standard rodent chow, Mucedola) and distilled water were available *ad lib* during the study and food was placed on the floor on day 18 postpartum since pups start to eat solid food around the age of 17 days (König and Markl, 1987). Food was also placed on the floor on the day of separation from the mother. Both the barren and the furnished cages were cleaned once a week during the rearing. The animal room had a controlled photoperiod (12h light: 12h dark, lights on at 04.20), the temperature was 19-21 °C and the relative humidity was 65-70%. During the night, the animal room was illuminated by infrared light to enable video recordings.

Mating took place when the weaned females were 16 weeks old and weighed 18,9-29,5g. Pairs or trios were formed by housing females from the same post-weaning cage with a male litter mate in standard wire-topped Makrolon II cages containing sawdust litter, a translucent red PVC nest box (MouseHouse, Techniplast) and tissue nesting material (1 double Kleenex sheet). At 17-18 days after mating and/ or when the females were visibly pregnant, the males were separated from the

females. The females were thereafter housed individually in standard wire-topped Makrolon II cages containing sawdust litter, a translucent red PVC nest box (MouseHouse, Techniplast), 1 cardboard tube cut in half and tissue nesting material (1 double Kleenex sheet) as can be seen in Picture 2. A total of five females reared in barren cages and six females reared in furnished cages were observed in the study. A summary of the different housing systems can be seen in Table 1.

Picture 1. Different housing systems for the females in the study during the rearing. Left: Furnished cage. Right: Barren cage. Food and water were placed on a wire-top.

Picture 2. The cage enrichment used during the maternal behaviour studies (Housing after separation from the male and throughout the lactation period).

Table 1. Different housing systems during the study.

Housing system	**Cage and contents**	**Females**
Housing during rearing		
Barren	Makrolon II (265x205x140mm) Sawdust.	S2AF1a, S2AF1b, S2BF1a, S3AF1b, S3AF1e
Furnished	Makrolon III (265x410x175mm) Sawdust, bedding sawdust, chew block, half Kleenex sheet, translucent red PVC nest box, cardboard nest box.	F2BF1b, F2AF1a, F2AF1b, F3AF1b, F4AF1a, F2AF1d
Housing after weaning		
	Makrolon II Sawdust, cardboard tube, absorbent paper.	All females
Housing during mating		
Housing after separation	Makrolon II Sawdust, translucent red PVC nest box, 1 double Kleenex sheet.	All females
	Makrolon II Sawdust, translucent red PVC nest box, cardboard tube cut in half, 1 double Kleenex sheet.	All females

2.2.2 Collected data & Observations

The females were weighed at mating and at the time of separation from the males. After giving birth to their first litter, the females and pups were weighed on day 4 or 5, between day 10 and 16 and before weaning (between day 19 and 23). The weighing always took place at the same time of day (12.00). When weighing the pups, all pups in one litter were weighed together as one. Therefore, an average weight per pup was calculated dividing the total weight with the number of pups in each litter. The mean number of pups per litter was calculated for the two different sample groups. A student's t-test was performed to see if it was any significant difference between the means.

The cages were filmed continuously from the day the females and males were separated to day 4 postpartum and for approximately 24 h at two more occasions after parturition, day 9-15 and day 18-22. Maternal behaviour was analysed using The Observer (Noldus Technologies) at six different occasions for each cage: 17.00-19.00 before and 07.00-09.00 on the days of weighing.

Summary of post-parturition filmings:

♦Observation One	Day 3, 4 or 5	17.00-19.00 and 07.00-09.00
♦Observation Two	Day 9, 10, 11, 12, 13, 14, 15 or 16	17.00-19.00 and 07.00-09.00
♦Observation Three	Day 18, 19, 20, 21, 22 or 23	17.00-19.00 and 07.00-09.00

Recordings were made with three or four cages at the same time and every cage was filmed in 30 s intervals. Behaviours and locations for the eleven females were continuously recorded for each cages every 30 s interval in the cases when the film was alternating between four cages. When the film was alternating between three cages, every fourth interval was skipped to make the total observation time 30 minutes in both cases. The ethogram (first structured by Weber, 2005) in Table 2 was used to score behaviours. Related behaviours were later merged for the statistical analysis (see Table 3). If the females built their nest in the nest box, Nest was scored as location.

Table 2. Ethogram

Ethogram -Categories of behaviours and behavioural definitions

Location (code)	**Definition**
Floor (1)	Mouse has two or more paws on the cage floor. Does not include when the mouse is in the nest (see Nest) or in the nest box (see Nest box).
Nest (2)	Mouse has >50% of the body in the nest area. Nest area defined as a structure made from paper and other loose parts from the cage and organised into a cluster in different shapes. Mouse scored as in nest when the nest is in the nest box.
Nowhere (3)	Code used when camera filmed other cages.
Nest box (4)	>50% of the mouse in or on the red PVC nest box.
Tube (5)	>50% of the mouse in or on the paper tube, does not include when the tube is a part of the nest (see Tube in nest).
Tube in nest (6)	>50% of the mouse in or on the paper tube when the tube is a part of the nest.
Cage top (7)	Mouse has one or more paws on cage top but no paw touching nest, nest box or floor.
Activity (code)	
Manipulating material (mm)	Mouse moves or lifts material (tube, paper) with mouth or paws.
Drink (dd)	Mouse has the mouth in contact with water pipe.
Eat (ee)	Mouse eats food from feeding site or elsewhere.
Digging (dg)	Mouse digs in the sawdust litter.
Not observed (nn)	Code used when camera filmed other cages.
Other (oo)	All other activities not described elsewhere or when activity not discernible.
Self grooming (sg)	Self-maintenance of pelage.
Pup activity (qq)	Mouse has visible contact with pups but activity cannot be distinguished. Does not include when mouse is still (see Nurse pups) or when pups not seen (see Nest activity).
Pup grooming (rr)	Maintenance of pup pelage by mouse.
Hidden (hi)	Mouse is hidden behind a structure and activity not seen. Does not include when the mouse is hidden in the nest (see Nest activity/ Nest still).
Nest active (aa)	Mouse movements in the nest but activity cannot be distinguished.
Nest still (ss)	Mouse is still (more than 3 s) in the nest but activity cannot be distinguished.
Exploring (xx)	Mouse is sniffing the components of the cage. Also includes locomotion which is not obvious exploring.
Bar circling (cb)	Mouse repeatedly traces a circle on the cage bars (more than twice).
Move/ lift pup (ml)	Mouse moves or lifts pup inside or outside the nest but without retrieving.
Nurse pup (np)	Mouse is still and has visible contact with one or more pups or mouse is visibly nursing.
Pup retrieval (rt)	Mouse retrieves pup from outside nest to the nest.
Tail chasing (tt)	Mouse is chasing its own tail in circle movements.
Mating (pp)	Mouse is doing any kind of mating behaviour (used in the Nestlets study).

Table 3. Summary of merged behaviours.

Merged behaviour	
Eat/ drink	Eat Drink
Nest building	Manipulating material- tube Manipulating material- paper
Nest behaviours	Pup activity Pup grooming Nest active Nest still Nurse pup
Locomotion	Exploring Tail chasing
Total in nest	Nest Tube in nest

A mean percentage of time spent on different activities and in different locations was calculated for the two groups of differently reared females to make comparisons. The statistical software SPSS was used to analyse data using multivariate analysis of variance (MANOVA). If the p-values for Pillai's trace were less than 0.05 it would mean a significant difference between the dependent variables. An ANOVA was performed to follow up the MANOVA (Field, 2005). A normality check was performed to see if the data was normally distributed. Comparisons between rearing conditions and between morning and afternoon were made for each of the three different observation times.

During the two-hour sequences when maternal behaviour was observed, the number of pups outside the nest was observed using instantaneous scan sampling every 15th minute (9 observations per two hour film). A mean percent of pups outside the nest per two-hour sequence of film was calculated dividing the sum of percent pups outside the nest per sampling time with nine.

2.3 Results

The mean number of pups per litter for the females reared in barren cages was 6,00 while the females reared in furnished cages got an average of 7,67 pups per litter. It was no significant difference between the means according to the student's t-test (df = 9, t= 1,30, p(0,05) = 2,26). The mean weights of the females and the pups are displayed in Figure 1.

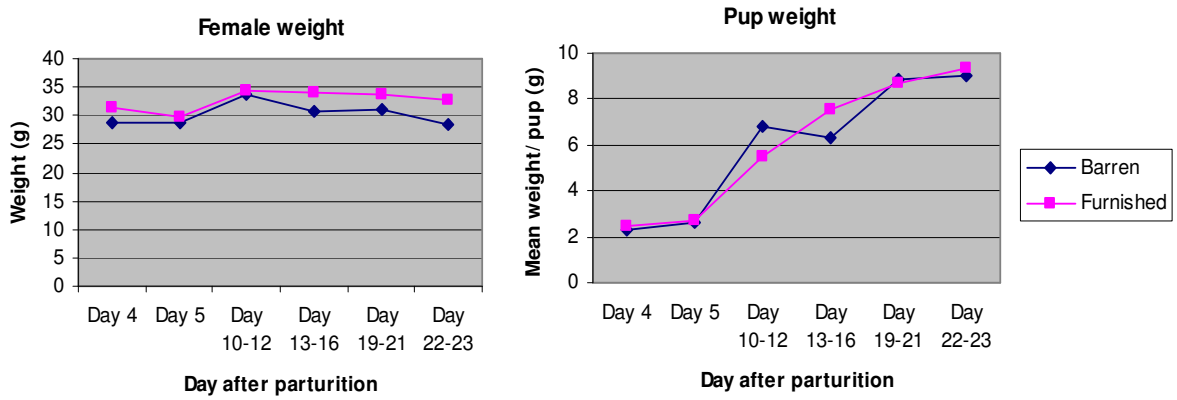


Figure 1. Left: A comparison of the mean weights for females reared in different housing systems when they have given birth to their first litter. Right: A comparison of the weight development for first litter pups to females reared in different housing systems.

The behaviours of eleven females exposed to one of two different rearing conditions were analysed between births and weaning of their first litter. Mean percentages of time spent on different activities during the three observations are shown in Figure 2 (for the females reared in furnished cages) and Figure 3 (for the females reared in barren cages). The females were more active (Digging, Self grooming, Eating and Drinking) and moved around more during the dark period. When it was difficult to distinguish what the female did, e.g. when she had her back towards the camera Other (oo) was scored as activity. Other was also scored as activity when the female was resting alone on the floor, on the nest box or in the nest.

Pup retrieval and Move/ lift pup only occurred once, therefore these behaviours were not further analysed. No abnormal behaviours were observed. When the female was eating at the same time as she was nursing the behaviour the female was actively performing was scored, in this case eating. An overview of significant differences between evening and morning activities are shown in Table 4. There were no significant differences between females reared in different housing systems.

Table 4. A comparison of differences between activities during evening and morning at a 0,05 significance level (ANOVA). Nsd = No significant difference.

Activity	**Observation One**	**Observation Two**	**Observation Three**
Digging	F=8,354; p=0,010	*Nsd*	*Nsd*
Other	*Nsd*	F=7,037; p=0,018	*Nsd*
Self grooming	*Nsd*	F=6,0153; p=0,027	*Nsd*
Hidden	*Nsd*	*Nsd*	*Nsd*
Eat/ drink	F=44,297; p<<0,0001	F=16,791; p=0,001	F=7,540; p=0,016
Nest building	F=10,123; p=0,005	*Nsd*	*Nsd*
Locomotion	F=26,079; p<0,0001	F=12,839; p=0,003	F=7,578; p=0,016
Nest behaviours	F=77,295; p<<0,0001	F=32,187; p<0,0001	F=10,141; p=0,007

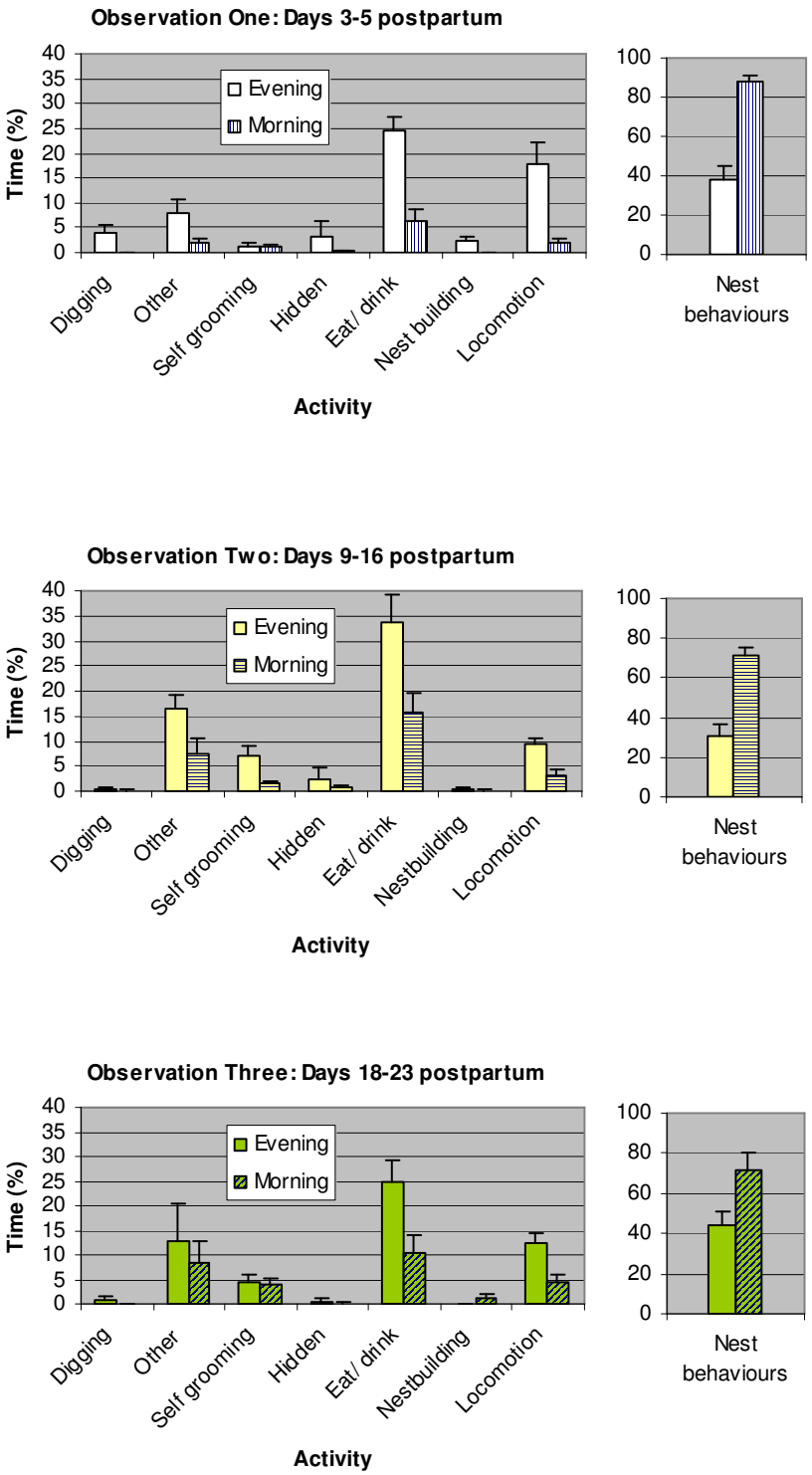


Figure 2. Mean time (+SEM) spent on different activities during evening (40min after lights off) and morning (2h 40min after lights on) for the females reared in furnished cages.

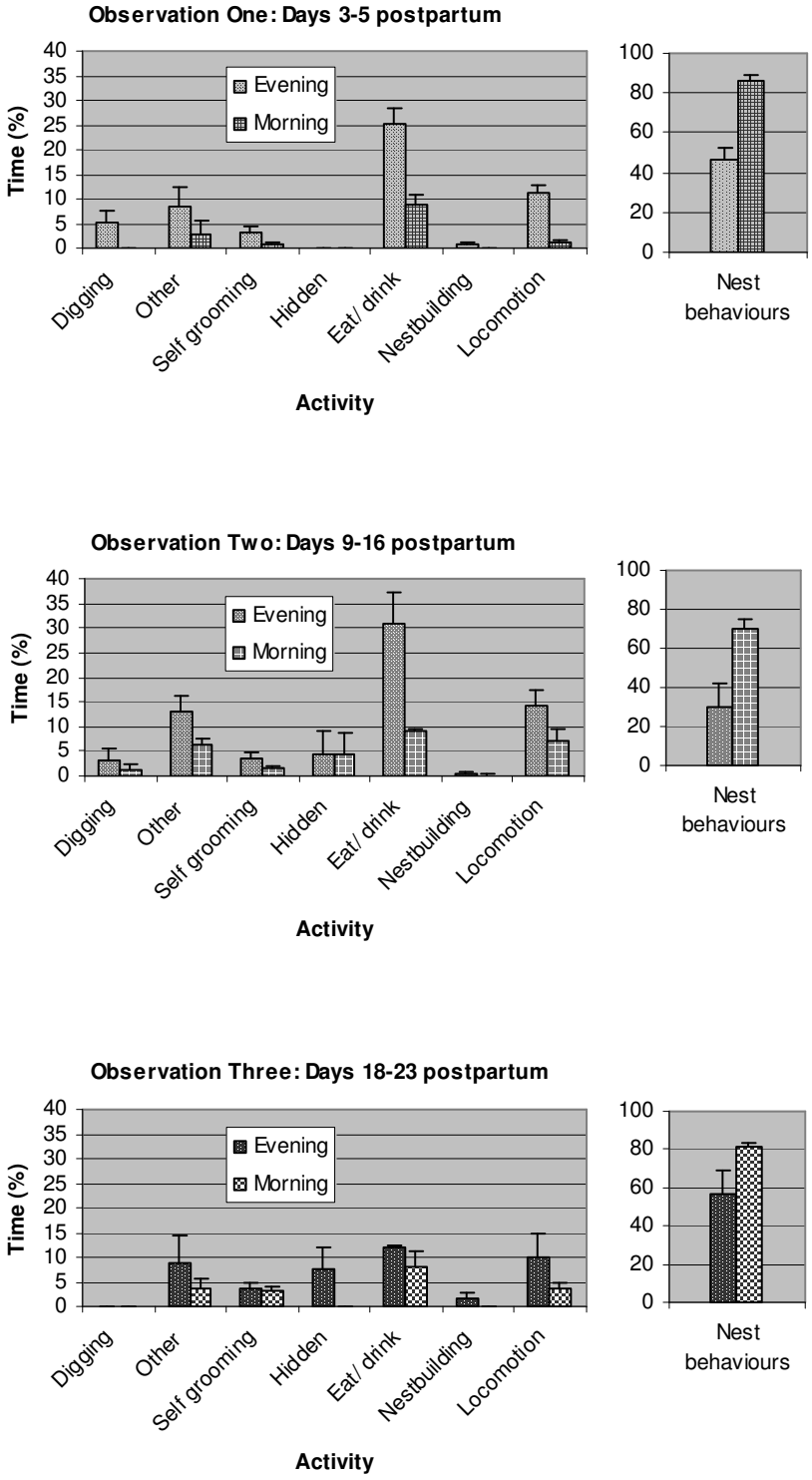


Figure 3. Mean time (+SEM) spent on different activities during evening (40min after lights off) and morning (2h 40min after lights on) for the females reared in barren cages.

The mean percentages of time spent in different locations during the three observations are shown in Figure 4. There were no significant differences between females reared in different environments. Significant differences between locations during the evening and morning are displayed in Table 5, the females spent more time in the nest during the light period. None of the females was ever seen climbing on the cage top.

During Observation One, one female reared in a barren cage and one female reared in a furnished cage had built their nests in the nest box. They still had their nests in the nest box during Observation Two when two other females (one from each rearing system) also had moved their nests into the nest box. Three females had their nests in the nest box during the final observations, the female reared in the barren cage who had the nest there from the beginning, the second female reared in a barren cage who moved her nest into the nest box before the second observation and one female reared in a furnished cage who had had her nest outside the nest box earlier.

Table 5. A comparison of differences between locations during evening and morning at a 0,05 significance level (ANOVA). Nsd= No significant difference.

Location	**Observation One**	**Observation Two**	**Observation Three**
Total in nest	F=64,820; p<<0,0001	F=27,323; p=0,0001	F=9,244; p=0,009
Floor	F=33,288; p<0,0001	F=24,832; p=0,0002	*Nsd*
Nestbox In	F=4,705; p=0,044	*Nsd*	*Nsd*
Nestbox On	F=9,578; p=0,006	*Nsd*	F=8,221; p=0,012
Tube	*Nsd*	*Nsd*	*Nsd*

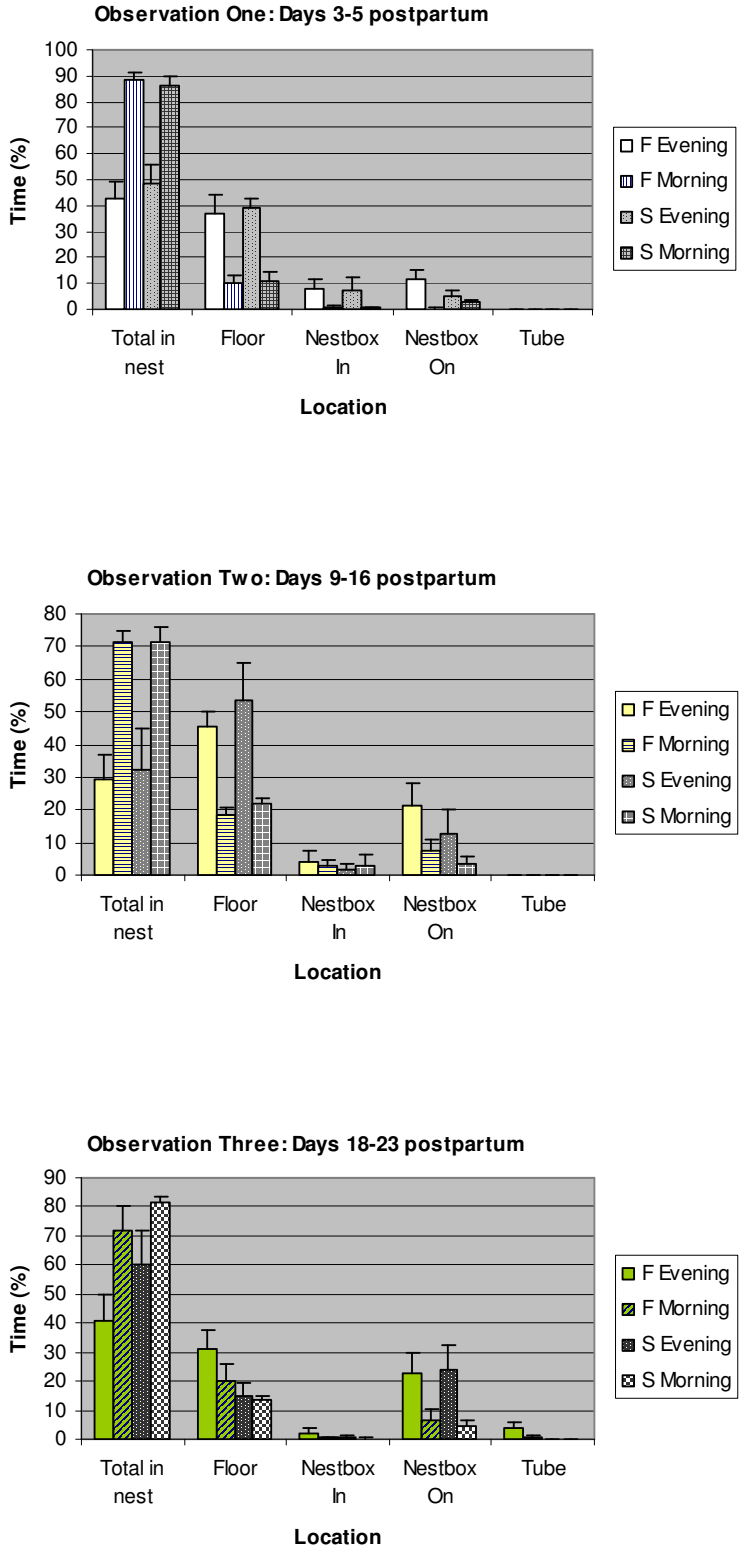


Figure 4. Mean time (+SEM) spent in different locations during dark/ light period (F= females reared in furnished cages, S= females reared in barren cages).

The mean percentages of pups outside the nest are displayed in Figure 5. Pups to the females reared in furnished cages came out of their nest earlier than the other pups.

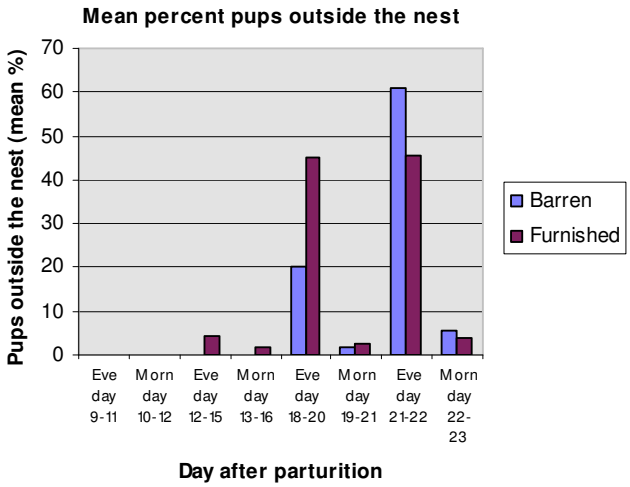


Figure 5. Percentage of pups to the females reared in furnished/ barren environment outside the nest on different days after birth. Eve = 40min after lights off, Morn = 2h 40min after lights on.

3 Experiment 2: Nestlets & Play Tunnel study

3.1 Aim

It is sometimes difficult to do behavioural observations are made due to complex nests and nests built in different places of the cage. This study was conducted to evaluate the possibility to use Nestlets and Play Tunnels from Datesand, Manchester as nesting material without blocking visibility and to see if it is possible to predict where the females will build their nests.

3.2 Materials & Methods

3.2.1 Animals and housing

Two 11 weeks old females (referred to as A and B) of strain Hfe -/- with C57BL/6J background bred at the IBMC were mated with 10-11 weeks old males (Hfe -/- with C57BL/6J background) in separate standard wire-topped Makrolon II cages (265 x 205mm, height 140mm) containing sawdust. Food (Mucedola, Italy) and water were available *ad lib* throughout the experiment. The experimental room was kept on a light: dark cycle (lights on at 05.00 and off at 17.35), temperature was 20-21 °C and relative humidity was 65-75%. To enable video recordings during the night, the animal room was provided with infrared light.

To evaluate Nestlets (Datesand, Manchester) as a nesting material, both cages were given one piece of approximately 1 g (cage A 1,3g, cage B 1g) on the day of mating. 2 days after mating, both cages were additionally given 1,1g of nesting material. 7 days after mating (first cleaning), both pairs were moved to new standard wire-topped Makrolon II cages containing sawdust and given new nest material; one piece of 2,4g Nestlets was given to both cages. 15 days after mating (second cleaning), the pairs were moved to standard wire-topped Makrolon III cages (265 x 410mm, height 140mm) containing sawdust. One cardboard tube (Play Tunnel 250 x 100 x 2mm, Datesand, Manchester) cut open and reduced in length to fit between the floor and the wire-top was put in one of the corners in both of the cages. Cage A was given one piece of new nest material (2,5g). Cage B's old nest was transferred to the Play Tunnel in the new cage and cage B was additionally given 2,5g of nesting material.

3.2.2 Collected data & Observations

The females were weighed seven times (maximum five days between weighing) before parturition. Males were weighed at mating and when cleaning took place. Pictures of the nests were taken when the females were weighed. The two cages were continuously recorded (with some unexpected breaks) from mating till day 5 postpartum, thereafter on the day before and the day after cleaning took place (day 14 and day 21).

To analyse where the mice spent most of their time and to evaluate how much time the animals spent on nest building and other activities The Observer (Noldus Technologies) was used to perform Instantaneous scan sampling (Martin and Bateson, 1993). Every 30 min from 12.00am twenty days before till 12.00pm the night before the females gave birth to their first litter the tapes were stopped for observations. The behaviours of both animals in one cage were summarised since it was impossible to distinguish the female from the male in the video recordings (the observations were therefore made distinguishing the animal to the left from the animal to the right no matter if it was the female or the male). Locations and activities monitored were: floor (1), total in nest (2), cage top (7), manipulating material (mm), eat/ drink (ee + dd), exploring (xx), digging (dg), other (oo), self grooming (sg), hidden (hi), nest active (aa), nest still (ss) and mating (pp). See the previously presented Ethogram in Table 2 for descriptions.

3.3 Results

The weights of the females and males are shown in Figure 6. Both females and males show the same weight pattern in both cages. Female A gave birth to two pups, one of each gender. Female B conceived nine pups, seven males and two females. The females became pregnant again after receiving their first litter; this can be seen as a weight increase on day 14 post-parturition.

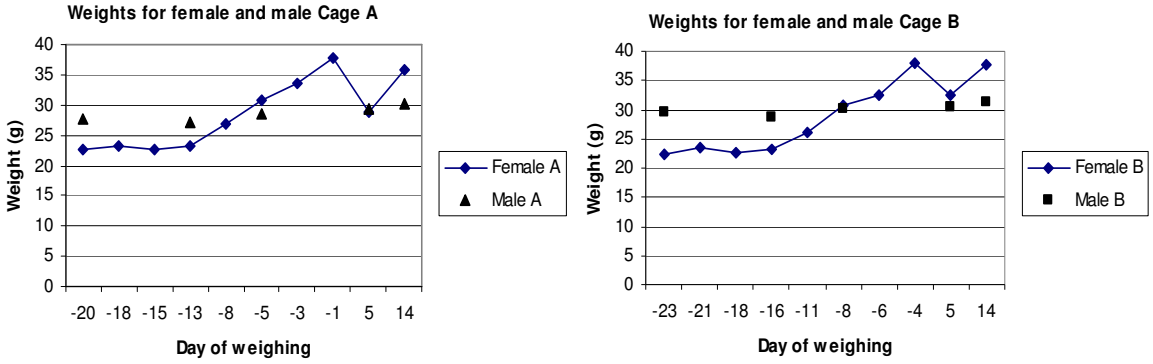


Figure 6. Weight development for the mice in the Nestlets & Play Tunnel study. Left: Cage A. Right: Cage B. Day 0 = day of parturition.

How much time the two pairs spent on manipulating material is shown in Figure 7. Due to technical problems (power cut in the laboratory), 56 observations are missing on day 12-10 pre-p for cage A and on day 15-13 pre-p for cage B. Cage A is also missing 15 observations during the interval 9-7 days pre-p while Cage B is missing 53 observations during day 12-10 pre-p. Both pairs used the provided pieces of Nestlets to build their nests. When provided with vertically placed Play Tunnels, the mice chose to build their nests inside the tunnel. The pair with less material (Cage A) gnawed

on the Play Tunnel and used the pieces that fell off for their nest. This was not seen in the pair with more nesting material. Instead, Cage B built a complex nest where they were able to hide under a duvet made of Nestlets from time to time. Picture 3 shows the two tubes used in the study. Complementary pictures from the Nestlets & Play Tunnel study can be found in Appendix A.

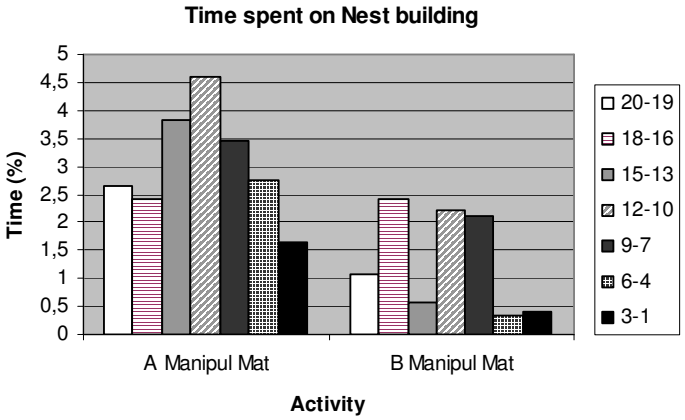
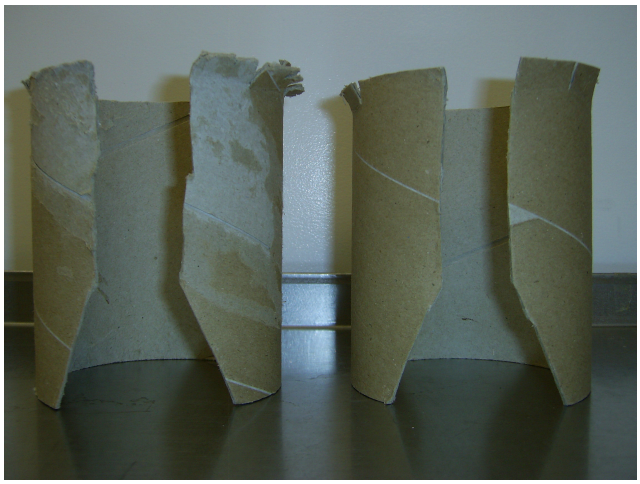


Figure 7. The sum of time spent on Nest building different days before parturition.

Times spent on other activities and in different locations are displayed in Appendix B.



Picture 3. Left: The Play Tunnel used in Cage A. The pair gnawed on the Play Tunnel and used the pieces that fell off for their nest. Right: The Play Tunnel used in CageB.

4 Discussion

The aim of this project was to compare maternal behaviour of females reared in different environments when they had received their first litters. A pilot study to evaluate the possibility to use Nestlets and Play Tunnels to furnish the cage was also conducted. During the experiments a number of methodological considerations having to do with the choices of observation methodologies and visibility in the cages had to be taken into account. Behaviours were compared during two intervals (one dark period forty minutes after light was turned off in the animal room and one light period two hours and forty minutes after the light was turned on) at three different occasions after parturition. The reason why these time intervals were chosen was that since mice are highly nocturnal (e.g. Latham and Mason, 2004) they would hopefully be in their most active state a while after light went off and presumably in the least active state (as in spending more time with the pups in the nest) during the light period.

The statistical analysis did not show any significant differences in maternal behaviour between females reared in different environments. Comparisons between behaviours during the dark period and the light period showed differences both in activities and where the mice spent their time. During the dark period the females were more active digging, eating/ drinking and moving around on the floor. When the lights were turned on, they spent significantly more time in the nest doing nest behaviours. König and Markl (1987) found similar differences in maternal care during morning and evening sessions. It is not very surprising that the females spent a lot of time with the pups during the observations. During the first weeks of life, pups are dependent on their mother for food, temperature regulation and stimulation (Latham and Mason, 2004). König and Markl (1987) also scored the behaviour Resting alone for females in their study, a behaviour repeatedly seen in this experiment suggesting an addition of this behaviour to the Ethogram used in maternal behaviour observations.

The weight curve for the females reared in furnished cages lies slightly higher than the curve for the females reared in barren cages. Every second measure point belongs to the same set of cages but the curves show the same pattern anyway. Still, it is interesting that the curve corresponding to the females reared in furnished cages shows a smaller decrease in weight than the barren curve in the end of the experiment. The data needs to be confirmed to draw any conclusions. The pup weight curves do not differ as much, the curve for the pups reared by females from the furnished housing lies slightly higher than the other curve. Big litters can lead to finite milk availability and hence affect a pup's adult weight (Latham and Mason, 2004). The mean number of pups per litter for the females reared in furnished cages was higher than for the barren females. Therefore, the finding that

both the females reared in furnished cages and their pups were heavier than the other sample group was a surprise. Offspring in large litters are generally lighter at birth and weaning (Weber and Olsson, 2006a) and rearing a large litter must be more demanding than rearing a small litter. The number of females and pups are probably too small to draw any conclusions; if one litter/female differs a lot from the rest it has a big effect on the curve.

The pups from the females reared in furnished environment came out of the nest earlier than the pups from the females reared in barren housing. This happened when the litters consisted of 8 and 10 pups and they followed their mother outside the nest for nursing. One possible explanation is that it is more difficult for big litter pups to get enough food from their mother. It is also common that pups accompany their mother during their first trips outside the nest (Latham and Mason, 2004).

Nest building is an important part of maternal behaviour. The nest helps the parents to regulate the temperature of the pups (Bond *et al.*, 2002). Results from the Nestlets and Play Tunnel study showed that both pair used the provided material to build their nests. Van de Weerd *et al.* (1998) conducted a study where they investigated the strength of preference for nesting material in laboratory mice and also established the fact that nesting material is important for the wellbeing of laboratory mice. The fact that the pair in cage A used pieces of the Play Tunnel for their nest is not surprising. They got less material than cage B and it has been shown in studies that mice often use a combination of nesting materials for nest building (Olsson and Dahlborn, 2002). Cage B's nest was probably complex enough without any additional material. During the 20 days nest building behaviour was observed, Cage A spent more time manipulating material than Cage B. They also gnawed on the Play Tunnel to get some additional material, probably because they were not satisfied with their nest and also the fact that nest building is a strongly motivated behaviour (Weber and Olsson, 2006a). Both pairs manipulated material from the day of mating until they gave birth to their first litter. There was a slight trend of a peak in nest building behaviour 15-7 days before parturition.

It was sometimes difficult to distinguish the behaviours due to reflections in the cage during the light period, bad angles or too far distance between the camera and the cage. That is one reason why some of the behaviours were merged before the statistical analysis. Another problem that arose was that the females chose to build their nests in different places. Sometimes making it easier to see nest behaviours while it was more difficult other times. This lead to the pilot study involving Nestlets and Play Tunnels, to see if it is possible to control where the females will build their nests, and so obtain better predictability and visibility for future experiments.

The use of Play Tunnels as a standard enrichment in a laboratory cage might make it a bit difficult when it comes to observing behaviours. One good thing was that both pairs used the Play Tunnel to serve as a nesting place. It facilitates filming when one can predict where the nest will be. On the other hand, to keep the Play Tunnel from being moved, the walls were as high as the cage and that made the nest area dark. It was also difficult to observe behaviours from above. New experiments are running with a different approach, the Play Tunnels are cut more open and filming is carried out from the side.

To choose a good sampling interval for instantaneous scan sampling is crucial for the outcome of the analysis. Instantaneous scan sampling is not accurate when precise values are wanted; it is more of estimation (Engel, 1996). It seemed relevant to collect data every 15th minute during the two-hour sequences to get an estimate of how many pups that were outside the nest. Whether or not instantaneous scan sampling gives a good picture of how much time mice spend on nest building can be discussed. As mentioned earlier, the sampling interval is crucial for the outcome of the analysis. The behaviour itself is also of interest. Some behaviours occur rarely and then only during a short time. The probability of catching the moment when that specific behaviour occurs is low (Engel, 1996). Nest building on the other hand usually lasts for many seconds, or even minutes, so instantaneous scan sampling seem to be an appropriate method for observing this behaviour. The behaviour was studied over a long period, 20 days, to be able to find whether there were any discernible differences in nest building during the time before parturition.

Choosing a satisfactory amount of Nestlets combined with the use of the Play Tunnel is also of interest. Too much material makes behavioural observations difficult to carry out because the nest becomes too enclosed. Provided with less material the mice may try to get pieces from the Play Tunnel as the pair in cage A did. The Play Tunnel was quite solid so it did not break because of the gnawing and the nest did not become as complex as the other nest. Still, one piece of Nestlets (ca 2,4g) might be too much and the mice are able to build a too complex nest complicating behavioural observations.

More studies need to be done when it comes to observing laboratory mice' behaviour. Firstly, for this particular project studying maternal behaviour, it is of interest to create a standard environment and standard procedures (e.g. when weighing and cleaning should be carried out) for filming and measuring behaviours to minimise variations. Secondly, to provide laboratory animals with environmental enrichment should be a rule in every laboratory for the wellbeing and welfare among laboratory animals. It can be stressful for laboratory animals to not be able to carry out normal and highly motivated behaviours and this might affect other studies performed on the animals. The time

the females in the first experiment spent in different environments during the rearing might have been too short to affect maternal behaviour, if there would be any difference due to environmental enrichment. Since the females were housed in similar enriched post-weaning environment from 19-23 days of age they might still have been in a sensitive period during that time. Hadley *et al*. (2006) found that enriched housing conditions up until 124 days of age was associated with lower levels of abnormal behaviours in deer mice. Finally, since the sample sizes in this study were quite small these findings may be considered as indications rather than definite results, in particular regarding the comparisons between groups.

5 Acknowledgements

I would like to thank Dr. Anna Olsson for giving me the opportunity to come to IBMC in Porto, Portugal to do my degree project and for being my supervisor. Further, I would like to thank Elin Weber for her valuable help and for all her answers to my questions and Robert Eriksson for help with pictures. Joana Santos for her help with the filmings and for taking care of the animals in the beginning of the experiment and professor Bengt Meyerson for reviewing my report. Another big thanks to all the people who made my stay in Portugal a pleasant one; Maria E, AnaMaria, Åsa, Andreia, Maria T, Reinhard and of course to my family and friends at home, I can always count on you.

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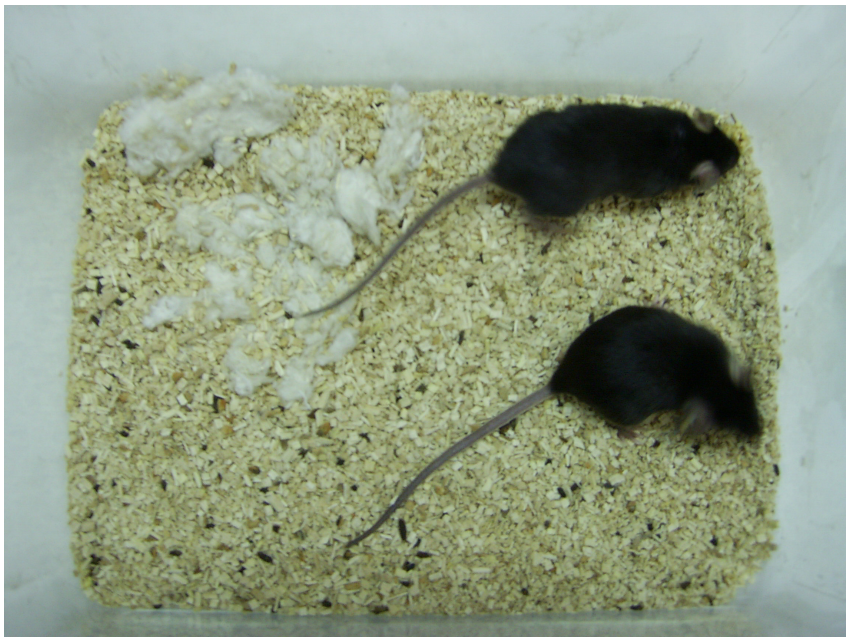
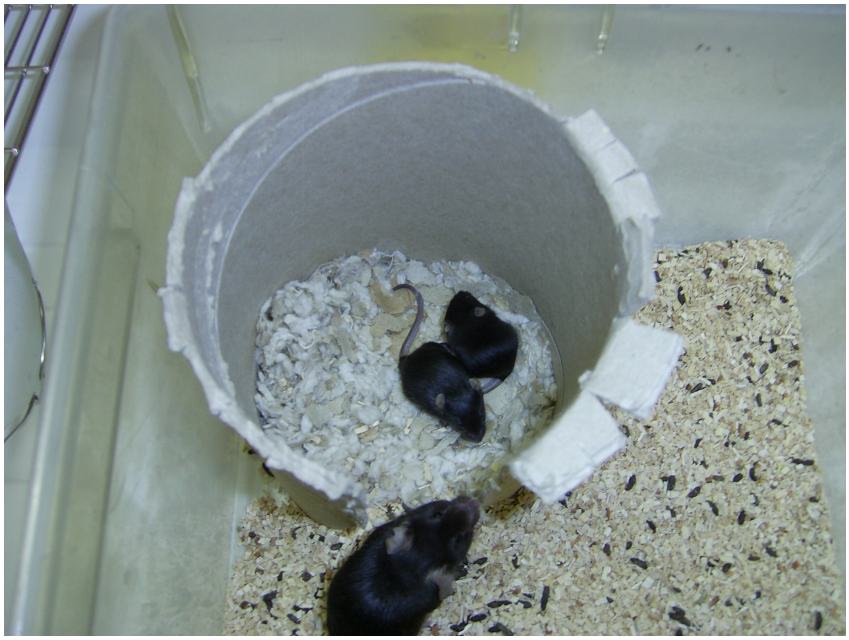
7 Appendix

7.1 A: Complementary pictures to the Nestlets & Play Tunnel study



One of the cages provided with 1g of Nestlets. It is difficult to predict where the mice will build the nest.

The Play Tunnel is fitted between the floor and the wire-top.

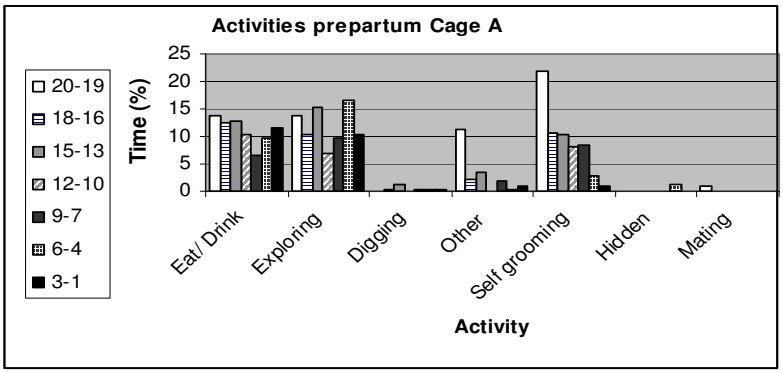


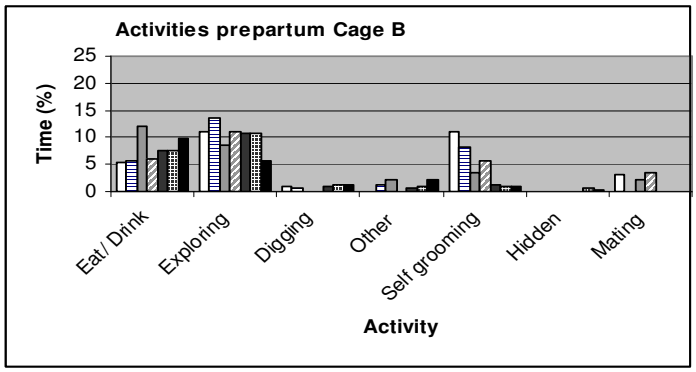
The pair in Cage A gnawed on the Play Tunnel and use pieces that fell off as additional nesting material.

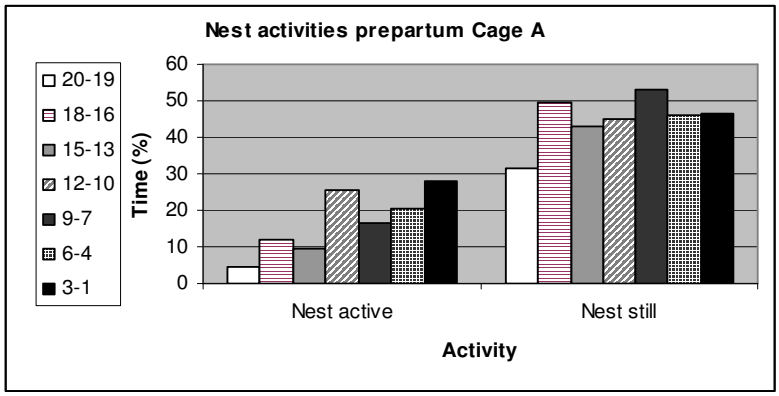
The pair in Cage B had enough material to make a duvet thus complicating behaviour observations.

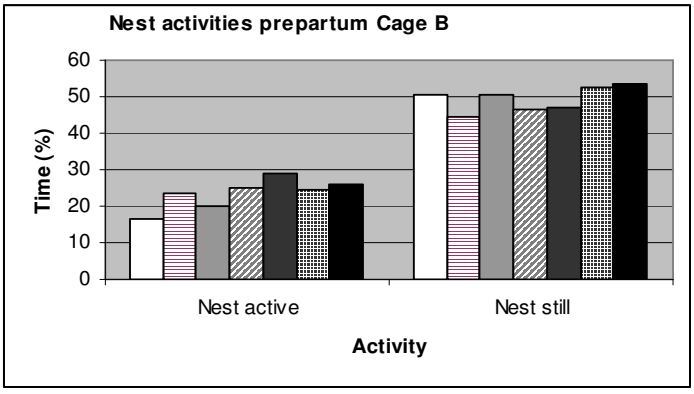
The tube was big enough for nine pups and their parents.

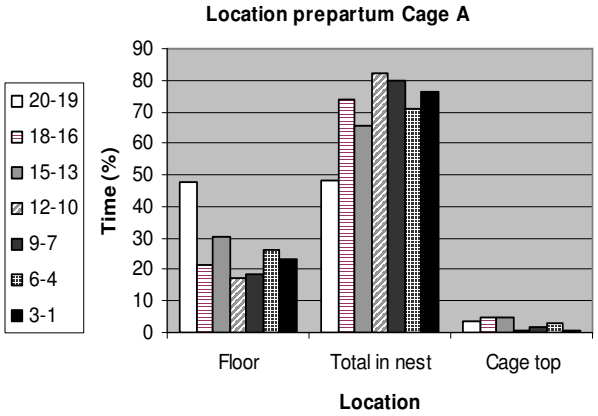
7.2 B: Activities and Locations in the Nestlets & Play Tunnel study

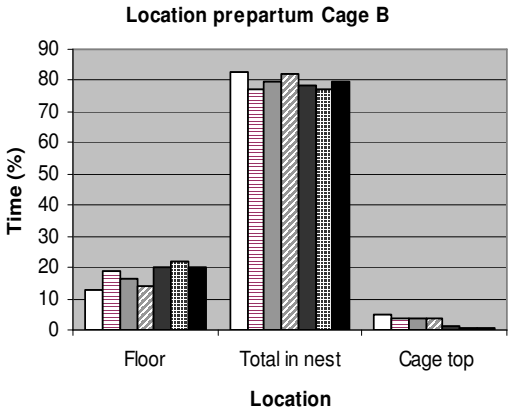









Location prepartum Cage A
20-19
18-16
15-13
12-10
9-7
6-4
3-1
Time (%)
90
80
70
60
50
40
30
20
10
0
Floor
Total in nest
Cage top
Location


Location prepartum Cage B
Time (%)
90
80
70
60
50
40
30
20
10
0
Floor
Total in nest
Cage top
Location