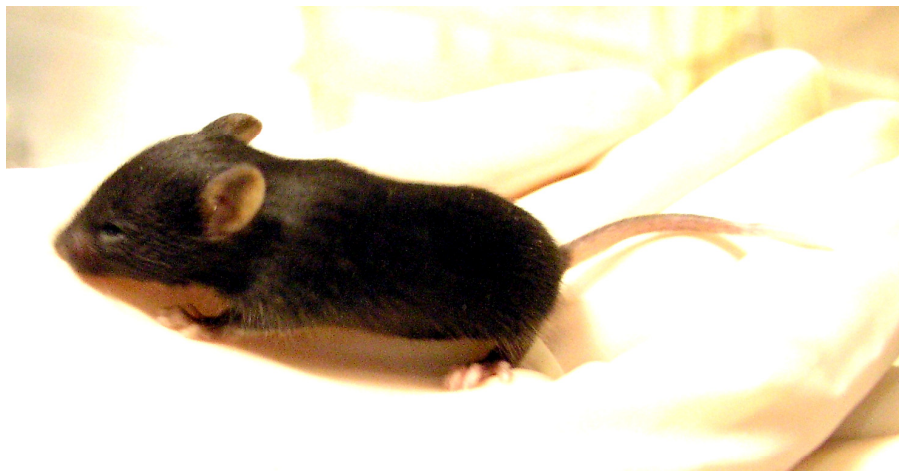




Maternal behaviour in the laboratory mice C57BL/6J housed in a furnished environment - a pilot study

*En pilotstudie av modersbeteende hos laboratoriemöss
(C57BL/6J) i inredda burar*

Elin Weber



**Sveriges Lantbruksuniversitet
Institutionen för husdjurens miljö och hälsa
Avdelningen för djurskydd**

Skara 2005

Studentarbete 53

***Swedish University of Agricultural Sciences
Department of Animal Environment and Health
Section of Animal Welfare***

Student report 53

ISSN 1652-280X

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Elin Weber

Examensarbete i Etologi, 20 poäng, Biologiprogrammet

Handledare: Dr. Anna Olsson
Laboratory Animal Science, Institute for Molecular and Cell Biology
Rua Campo Alegre 823, 4150-180 Porto, Portugal

Biträdande handledare: Prof. Linda Keeling
Department of Animal Environment and Health, Section of Animal Welfare
Swedish University of Agricultural Sciences,
Box 7038, 750 07 Uppsala

Abstract

Maternal behaviour covers a wide range of behaviours and is one important factor that can increase survival. Behaviours such as nest building, nursing, licking, lifting or moving pups, retrieving, and protection of the young are often included when measuring maternal behaviour. Commonly, observations of maternal behaviour are made on mice housed in barren cages. These cages contain nothing but sawdust and do not provide the animals with the opportunity to hide, to build nests or to make burrows. Mice are highly explorative animals and the barren environment thus restrains the animals from performing many natural behaviour patterns. In the present study, maternal behaviour was observed in the inbred strain C57BL/6J mice, housed in a furnished environment. In this study “furnished” refers to larger cages that, except from sawdust, also contained nest material, nest box, a paper tube and a chew block. The following comparisons were made: Effect of days - comparing prepartum/postpartum/day three postpartum; Effect of disturbance - comparing day three and four postpartum (before and after a pup retrieval test); Difference between Day and Night – comparison between light/dark period (on day three and four postpartum). Besides behavioural observations, the present study also aimed to investigate the best possible way of providing cage furniture, while maintaining the possibility to carry out behaviour observations. The results from the behavioural observations show that females spend significantly more time on nest behaviours after parturition compared to prepartum day and day three postpartum. Furthermore, they spent significantly more time self-grooming before parturition than postpartum and day three postpartum. There were no differences found in the behaviour following disturbance due to a pup retrieval test. Neither were there any differences found between behaviour during the light and dark period on day three or four after parturition. Since mice build complex nest structures of available material in the cage, observing maternal behaviour in furnished cages is a difficult task. The females in the present study were filmed from four different views, all which had different advantages. Depending on which behaviours that are of interest, one must choose to film from a view that gives best possibility to observe the behaviours of current interest.

Introduction

Reproductive behaviour

The wild house mouse is an opportunist well known for its fast reproduction and adaptive ability, and it has occupied several different habitats all over the world (Bronson, 1979). The social organization may vary between different mouse populations. Bronson (1979) described two common types: the commensal and the feral populations.

Commensal populations live in territories with stable and plentiful food supply and a population density of up to 10 mice/m². Feral populations are less dense with spatial, temporal and social instability, and are found in environments with seasonally unstable food supply. Commensal populations live in territories with a single dominant male, a few subordinate males and several breeding females with offspring, while feral populations are typically unstable with a high turnover rate.

Commensal house mice show no difference in breeding intensity throughout the year, compared to feral mice, which according to Berry (1970) have definite breeding seasons. However, Bronson (1979) claims that they are not obligatory seasonal breeders and breed all year in temperate zones. Probably the capacity of continuously and rapid breeding is only inhibited by environmental factors (Bronson, 1979).

Under favourable conditions, female house mice are sexually mature around the age of 6-8 weeks. They are short-cycling, with an oestrus cycle that varies from four to six days (Berry, 1970; Bronson *et al.*, 1966), have a spontaneous ovulation and produce many large litters (Bronson, 1979). The length of individual cycles vary however, depending on strain, season, diet and environmental factors (*e.g.* Baumans, 2004). The strain C57 has an average length of 5,4 days in February and 5,8 days in March (Bronson *et al.*, 1966).

Fertilization is possible for about 10-12 hours after ovulation and the gestation period lasts for 19-21 days (Berry, 1970). Parturition usually takes place during the night, and is immediately followed by *post partum* oestrus with ovulation 12-18 hours after giving birth (Berry, 1970).

At birth the young are hairless, deaf, blind and weigh approximately one gram (Berry, 1970) and they are fully dependent on their mother for nutrition (König & Markl, 1987). The pups are fully furred around the age of 10 days and they open their eyes between day 12 and 14 after birth (Fuchs, 1981). At the age of 17 days the pups start to eat solid food (König & Markl, 1987), and at approximately 21 days after birth weaning takes place (*e.g.* Fuchs, 1981). At weaning the mice weigh around 10 g, depending on litter size (Berry, 1970).

Maternal behaviour

The term "maternal behaviour" covers a wide range of behaviours and is one important factor that can increase the probability of survival of a population (Cohen-Salmon, 1988). Causey and Waters (1936) were the first to demonstrate the many phases included in parental care in mammals (Cohen-Salmon, 1988). Under parental care they included nest building by one or both parents, incubation, feeding behaviours (nursing, carrying and storage of food), protection of the young (*e.g.* by hiding, warning or carrying young), and training of young. Capabilities to feed or adopt young within and across species, abandonment and devouring of young were also included, but classified by the authors as aberrations.

The house mice in the laboratory

In some areas the wild house mice are considered agricultural pests. They also destroy wood, clothing and contribute to the spread of disease (Walker & Nowak, 1999; Morton, 2002). At the same time, selectively bred mice are used as experimental organisms in thousands of

laboratories all over the world every year (Silver, 1995). Today, there are hundreds of different inbred strains and substrains (Silver, 1995), all bred to fit the special needs in a specific research area.

Mice are active and highly explorative animals and they construct elaborate nests and burrows. These behaviours are characterized by a strong motivation and are still present in the laboratory mice (reviewed in Olsson & Dahlborn, 2002). In the laboratory however, mice are kept under very unnatural conditions. In general, they are housed in small cages containing nothing but sawdust (Olsson & Dahlborn, 2002). This housing condition does not provide the animals with the opportunity to hide, to build nests or to make burrows and thus restrain the animals from performing many natural behaviour patterns (Jennings *et al.*, 1998).

One way of improving housing conditions for laboratory animals is to provide them with a furnished environment (Baumans, 2004) containing for example nest material, paper tubes, nest box etc. This gives the animals an opportunity to control their environment to a higher degree than in a housing system where only sawdust is provided. Today, it is generally accepted that a furnished environment improves the animals well-being (Baumans, 2004).

Observing maternal behaviour

Since house mice are largely nocturnal and often live in burrows (Olsson *et al.*, 2003) it is difficult to make observations of their behaviour in the natural habitat. There are also few studies conducted with the specific purpose of viewing the standard maternal behaviour of the house mice (Ostermeyer, 1981). Most studies concentrate on a specific item in the complexity of the behaviours, such as maternal aggression (*e.g.* Maestripieri & Alleva, 1990), communal nesting (*e.g.* Saylor & Salmon, 1971), infanticide (*e.g.* Soroker & Terkel, 1988) etc..

Pup retrieval tests are often used to measure maternal behaviour (see *e.g.* Gandelman, 1973; Noirot, 1974; Hennessy *et al.*, 1980; Cohen-Salmon *et al.*, 1985; Pandey & Pandey, 1989; Pardon *et al.*, 2000), and it is considered to be highly characteristic for the muridae to retrieve pups if they are absent from the nest (Cohen-Salmon *et al.*, 1985).

Commonly, observations of maternal behaviour in the laboratory are made on mice housed in barren cages. (see *e.g.* Gandelman, 1973; McCarthy & vom Saal, 1985; Maestripieri, 1989; Elwood *et al.*, 1990; König, 1994; Manning *et al.*, 1995). Even though the mother spends much time curled around the young (Ewer, 1968) and it might be difficult to observe all pups, the behaviours self-grooming, licking pups, nursing, and lifting or moving pups can be observed (see *e.g.* Ostermeyer, 1981). Mice have a strong motivation to build nests (see *e.g.* Cohen-Salmon, 1988), and nestbuilding increases during pregnancy (Schneider *et al.*, 1982). When housed in furnished cages with access to nest material, complex nest structures are built. This makes behavioural observations more difficult and the above-mentioned behaviours much more hard to distinguish.

Aim

The aim of this study was to describe the maternal behaviour of the inbred strain C57BL/6J mice, housed in furnished cages. In this study “furnished” refers to larger cages that, except from sawdust, also contain nest material, nest box, a paper tube and a chew block.

The original aim of the study was to study the effect of housing conditions on maternal behaviour. However, several problems arising during the study made us realise a pilot study was needed, and the present study was converted into this pilot study, in which the best possible way of providing cage furniture while maintaining the possibility to carry out behaviour observations was explored.

Material & Method

Animals and housing

The study was conducted at the Institute for Molecular and Cell Biology in Porto, Portugal. It was divided into two parts, the first from September to December 2003, and the second from April to June 2004. Animals used were the inbred strain C57BL/6J, bred in the laboratory. The animal room was kept on a 12h light: 12h dark cycle with lights on at 07:30. Temperature was maintained at 21-23°C and relative humidity was 70-100%.

A total of 12 females were used in the study (see Table 1). Ten females were housed in furnished cages in standard wire-topped Makrolon III cages (265 x 410 mm, height 175 mm) containing sawdust, one cardboard tube (100 mm long, 45 mm diameter), one chew block, one sheet (2,3 g) of absorbent paper (Renova SA, Torres Novas, Portugal), and a translucent red PVC nest box (MouseHouse, Tecniplast) or a cardboard nest box (Des Res., Lillico). In order to film from above the top of the cardboard nest box was cut (see picture A, appendix 1). There were also two females used housed in barren cages in standard wire-topped Makrolon II cages (265 mm x 205 mm, 140 mm high), containing nothing but sawdust. These animals were used as reference animals since all behaviours were much more easy to observe in the barren cage. Food (standard rodent chow, Harlan Iberica) and water was provided *ad libitum*, and besides this the females in the furnished cages were given extra grains (mixture of corn and wheat) once a week. Both the furnished and the barren cages were cleaned once a week, except for the time around parturition when the females were left undisturbed.

Table 1. Different housing systems used in the study, and the number of females housed in each system.

N females	Housing	Note
6	Furnished: Type III cage, nest material, PVC nest box, paper, paper tube, chew block.	
4	Furnished: Type III cage, nest material, cardboard nest box, paper, paper tube, chew block.	One female excluded from before/after disturbance (day three and four postpartum) due to infanticide.
2	Barren: Type II cage with sawdust only.	One female excluded from the study due to infanticide.

Table 2. Different recording views used in the study, and the number of filmings from each view (see Appendix 1 and 2).

Day	Housing	N Filmings	Recording view	Note
Pre/post-partum	Furnished	-	Front short side	Two females not recorded pre- and postpartum.
		3	Front long side	
		3	From above	
		1	Close view of part of cage	
Day three	Furnished	2	Front short side	One female infanticidal and therefore not observed during day three and four.
		1	Front long side	
		2	From above	
		3	Close view of part of cage	
Day four	Furnished	1	Front short side	
		3	Front long side	
		2	From above	
		2	Close view of part of cage	
Pre/post-partum	Barren	-	Front short side	One female infanticidal and therefore excluded from the study.
		2	Front long side	
		-	From above	
		(2)	Close view of part of cage	
Day three/ day four	Barren	-	Front short side	
		2	Front long side	
		-	From above	
		-	Partly hidden	

Procedure

All females were mated in pairs at the age of approximately 10 weeks. They were separated from the male and housed singly when visibly pregnant (around two weeks after mating). The cages were kept in the racks (for side view filming only) or on the floor (when filmed from above) during recording. When moved out of the rack, females were habituated in the new location for one hour prior to filming. The behavioural observations were divided into two parts: before/after parturition and before/after a disturbance.

Infanticide occurred once in the barren and once in the furnished cages. Infanticide in the barren cage occurred on day one postpartum and the female was therefore excluded from the whole study. Infanticide in the furnished cage occurred on day three; this female was therefore only excluded from the observations before/after disturbance. Since there was only one female from the barren cage, no comparisons were made between barren and furnished environments, but we decided to include her as a reference animal.

Video recordings

The video recordings started when the female was separated from the male and housed singly. This was done in order to observe the exact time of parturition, and after that observe the behaviour of the female 12 hours pre- and 12 hours postpartum. The second part of the recordings was made 24 hours prior and 24 hours after a pup retrieval test, in order to observe the behaviour before and after this disturbance.

Parturition

By recording the time before and after parturition the time of birth could be observed. The exact time of birth was detected when the female was first seen standing in a characteristic birth position (Ewer, 1968). When this was not seen, the time was assessed as falling in the middle of an interval of nest activity between the last observation of obvious pregnancy and the first when the female was obviously no longer pregnant. A total of ten hours of film was observed to detect the exact time of birth for all females.

Observations

Behavioural observations were made using the Observer Software (Noldus Technology). Behaviours were scored with continuous recording for 2,5 min every half hour for a total time of 12 hours. In most cases recordings were made with more than one cage at the same time using a switcher, giving an interval of 30s recording of each cage. Therefore, every sampling part was divided into 30 second intervals, with a total of 10 min sampling period to obtain 2,5 min observation time (in the case with four cages). A total of approximately 65 hours was used for behavioural observations during pre- and postpartum, day three and day four. The observed behaviours are shown in the ethogram below.

Ethogram	Categories of behaviours recorded, and behavioural definitions.
Behaviour category and code	Definition
Location	
<i>Cage top (CT)</i>	One or more paws on cage top but no paw touching nest, nest box or floor.
<i>Floor (FL)</i>	Two or more paws on the cage floor except the nest, the tube and the cage box.
<i>Nest (NE)</i>	>50% of the body in nest area. Nest area defined as a structure made from paper and other loose parts from the cage and organized into a cluster in different shapes. When paper is missing, area defined by a cavity in the sawdust where the animal spends most time resting.
<i>Chewblock (CB)</i>	Sitting or standing on top of the chew block.
<i>Nest box (NB)</i>	Animal on or in the nest box (red PVC nest box) with >50% of the body.
<i>Tube (TU)</i>	Animal inside or on the paper tube with >50% of the body, does not include if the tube is a part of the nest (see Nest).
<i>Tube in nest (TN)</i>	Animal on or in tube with >50% of the body, when tube is a part of the nest.
Activity	
<i>Bar circling (BC)</i>	Repeatedly tracing a circle on the cage bars (movement repeated more than twice).
<i>Circling (CC)</i>	Chasing own tail in circle movements.
<i>Digging (DG)</i>	Digging in the sawdust litter.
<i>Eat/drink (ED)</i>	Intake of food or water (from feeding site or elsewhere).
<i>Exploring (XX)</i>	Sniffing the components of the cage e.g. sawdust substrate, cage equipment, cage walls, airborne odours or the environment external to the cage by sniffing through cage bars. Also includes locomotion without obviously exploring.
<i>Hidden (HI)</i>	Animal is behind any structure or out of camera view, and not seen. Does not include when animal is hidden in the nest by nest structure (see Nest activity and Nest still)
<i>Manipulate material (MM)</i>	Moving or lifting material (chew block, paper, tube) with mouth or paws.
<i>Nest activity (NA)</i>	Movements in the nest, but its activity can not be distinguished.
<i>Nest still (NS)</i>	In nest, not moving, but activity can not be distinguished.
<i>Other (OT)</i>	All other activities not described elsewhere.
<i>Pup activity (PA)</i>	Visible contact with pups but activity can not be distinguished. Not when mother is still (see Nurse pups) or pups not seen (see Nest activity).
<i>Pup grooming (PG)</i>	Maintenance of pup pelage by mother.
<i>Move/lift pup (ML)</i>	Moving or lifting pup inside or outside nest but without retrieving.
<i>Nurse pup (NP)</i>	Visible contact with one or more pups, mother still or visibly nursing.
<i>Pup retrieval (PR)</i>	Retrieving of pup outside nest to the nest.
<i>Self grooming (SG)</i>	Self-maintenance of pelage.

Pup retrieval test

Nine females (six from part one and three from part two) with litter from furnished and one from the barren cage were used. The test was carried out at 1600 h on day four after parturition (date of birth day 0). The female was removed from the home cage and placed in a cage containing nothing but sawdust. The pups were then gently moved from the nest, weighed and placed back in the home cage in a definite position from the nest. The position of the pups was chosen with consideration of the difference in cage size between barren and furnished cages. The purpose of this was to always place the pups with the same distance from the nest. In the second part of the study the females were also weighed before being placed into the empty cage. After placing the pups in their position the female was returned to the home cage and placed in the nest. The test ended when all pups had been retrieved, or, if

this did not happen, after 15 minutes. The test was video recorded through a camera placed over the test cage, and time for start and end of the below described behaviours were measured from the video recordings (see Table 3).

Table 3. Definition of behaviours measured in the pup retrieval test

<i>Direct complete (DC)</i>	Female retrieves pup from original (primary) position and places it in the nest, also includes if the female leaves the pup but retrieves the same pup again within 30s and places it in the nest.
<i>Indirect complete (IC)</i>	Female retrieves pup from other than original position and places it in the nest.
<i>Incomplete (I)</i>	Female retrieves pup from the original position or elsewhere but does not place it in the nest, also includes if the female leaves the pup but retrieves the same pup again within 30s and still does not place it in the nest.
<i>Manipulate nest (MN)</i>	Female manipulates pup within the nest.
<i>Manipulate outside nest (MO)</i>	Female manipulates young outside nest without retrieving.
<i>Sniff young (SY)</i>	Female sniffs at young without retrieving or manipulating it.

Statistical analysis

For the statistical analysis, related behaviours of the ethogram were merged, as described in Table 4. Note that some behaviour patterns have been included in several groups.

Table 4. Summary of merged behaviours

Merged behaviour	Behaviour category
Nest building	Manipulate Paper Manipulate Tube Manipulate Chewblock
Pup Activities	Pup activity Groom pup Pup retrieval
Nest behaviours	Pup activity Groom pup Nest active Nest still Nurse pup Pup retrieval
Locomotion	Exploring Circling Bar circling
Stereotypes	Circling Bar circling
Tube	Tube in Tube on
Totalnest	Nest Tube in nest

The mean percentages of time from the behavioural observations were used to make comparisons. The following comparisons between behaviours were made:

- Effect of days - comparing prepartum/postpartum/day three;
- Effect of disturbance - comparing day three and four (before and after the pup retrieval test);
- Difference between Day and Night – comparison between light/dark period (on day three and four).

Data analyses were made using analysis of variance (ANOVA) in the statistical software SPSS. Bonferroni corrections were applied for multiple comparisons. When the data were not normally distributed the values were transformed.

For the pup retrieval test, time for start and stop of the behaviours were noted using the video time. The total time for retrieving and the mean time for retrieving each pup were calculated in Excel.

Results

Parturition

Time for parturition was estimated for eight females in the study (see Table 5). The length from first mating opportunity to birth varied from 19 to 29 days. Excluding the high length of 29 days, this gave an average length of 21 days. Six females gave birth during the dark period, five of them between 02:00 and 05:30. Only two females gave birth during the light period, this between 08:00 and 10:00.

Table 5. Time of birth and number of pups for eight females in the study.

Female	Time of birth	Number of pups
SS6B	03:40	8
FF5A	05:10	6
FF6A	21:30	7
FF6C	03:10	8
FF7A	02:00	8
FF9A	09:40	Infanticide
FF9B	04:30	3

Behavioural observations

The mean percentages of time spent on different activities and locations for the female in the barren housing are shown in Figures 1 and 2.

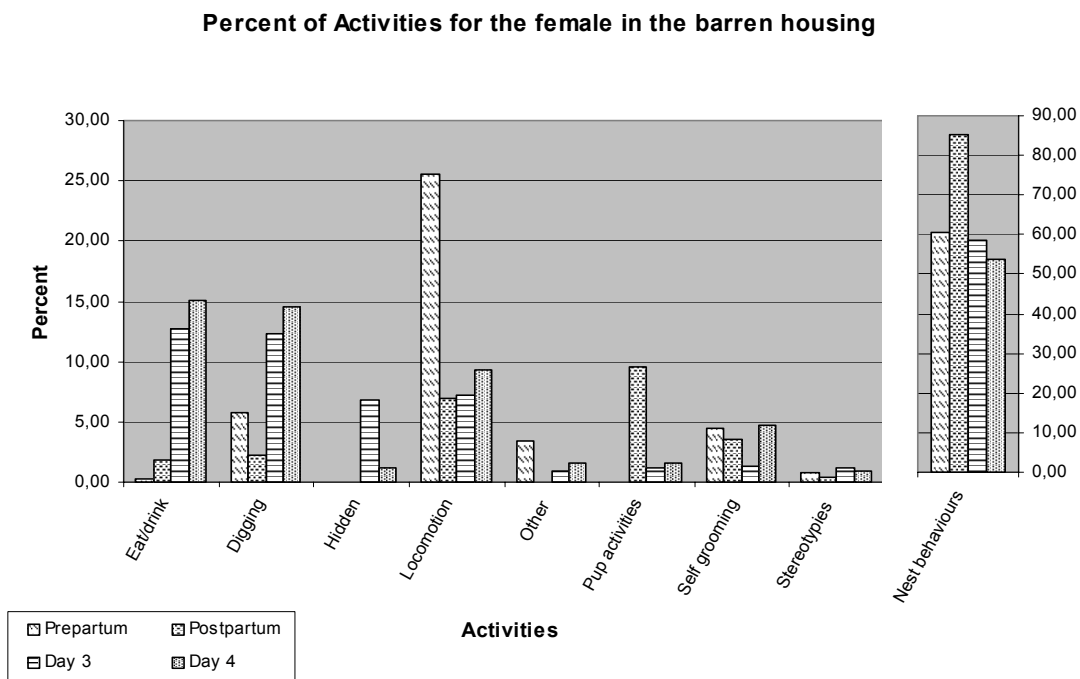


Figure 1. Percent of time spent on different Activities for the female in the barren housing.

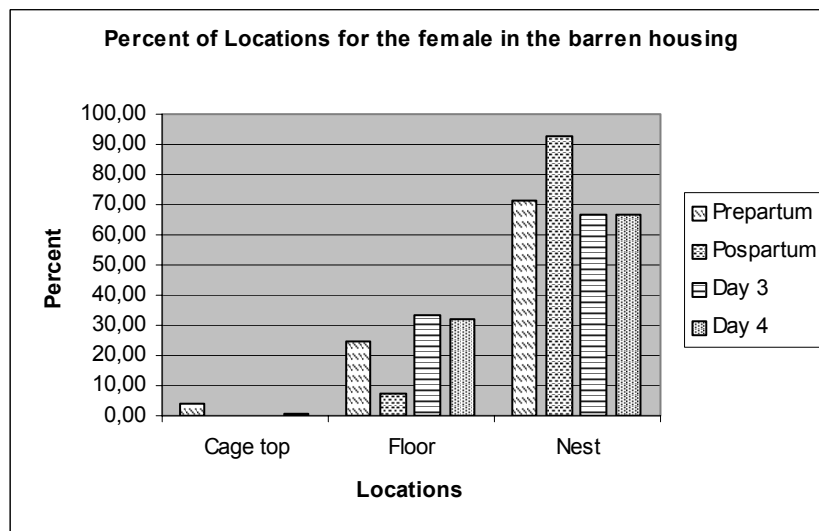


Figure 2. Percent of time spent on different Locations for the female in the barren housing.

For the females in furnished housing, results from the behavioural observations were divided into effect of days, effect of disturbance and differences between day and night (see Table 6 for an overview of results).

Table 6. Results from behavioural observations for the females in furnished housing. Reported differences were significant at the 0,05 level (ANOVA).

Behavioural observation	Behaviours	Differences	Values
Effect of days	Activity	Nest behaviours	Postpartum - Prepartum Postpartum - Day three
			F=34,456; P<0,000
		Locomotion	Prepartum - Postpartum
			F=5,812; P=0,017
	Self-grooming	Eat/drink	Day three - Postpartum
			F=5,727; P=0,009
		Digging	Day three - Postpartum
Effect of disturbance	Locatio		F=5,658; P=0,008
		Prepartum - Postpartum	F=5,817; P=0,003
		Prepartum - Day three	F=5,817; P=0,020
Difference between day and night	Nest	Floor	Prepartum - Postpartum
			F=24,701; P=0,005
		Day three - Postpartum	F=24,701; P=0,000
		Postpartum - Prepartum	F=22,456; P=0,003
		Postpartum - Day three	F=22,456; P=0,000
			F=22,456; P=0,000

Effect of days

After parturition the females spent significantly more time on nest behaviours compared to prepartum and day three (ANOVA, F=34,456, P<0,000). They spent significantly less time on locomotory behaviours after parturition compared to prepartum (ANOVA, F=5,812, P=0,017). Females spent significantly less time eating/drinking and digging after parturition than on day three (ANOVA, F=5,727, P=0,009; F=5,658, P=0,008). Before parturition they spent significantly more time self-grooming than on postpartum day and day three (ANOVA, F=5,817, P=0,003; 0,20) (see Figure 3).

After parturition the females spent significantly less time located on the floor and more time in the nest than before parturition and day three (ANOVA, F=24,701, P 0,005; P<0,000. F=22,456, P 0,003; P<0,000) (see Figure 4).

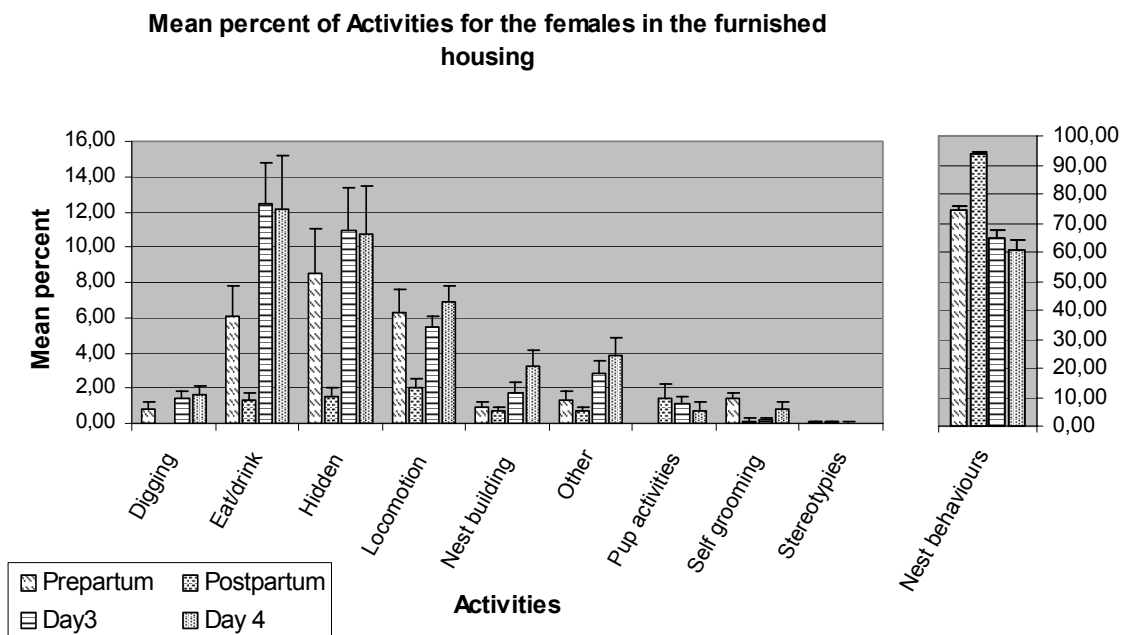


Figure 3. Mean percent of time spent on different Activities during Prepartum, Postpartum, Day 3 and Day 4, for the females in the furnished housing.

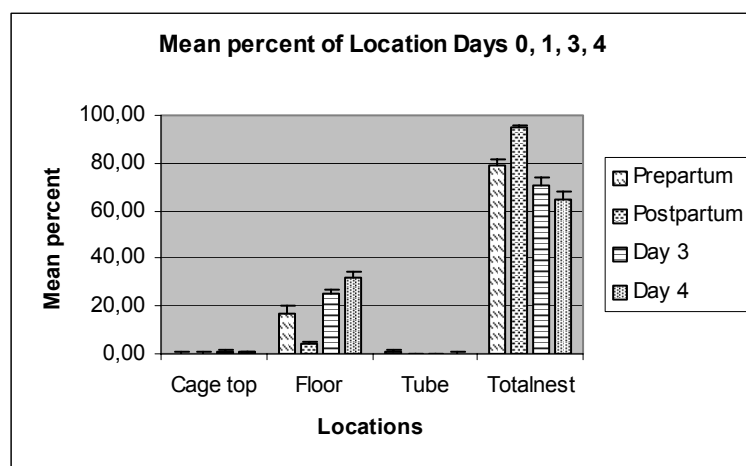


Figure 4. Mean percent of time spent on different Locations during Prepartum, Postpartum, Day 3 and Day 4, for the females in the furnished

Effect of disturbance

There were no significant differences in activity or location before and after the disturbance (see Figure 3 and 4).

Difference between Day and Night

There were no significant differences in activity or location between day and night during day three and day four (see Figure 5 and 6).

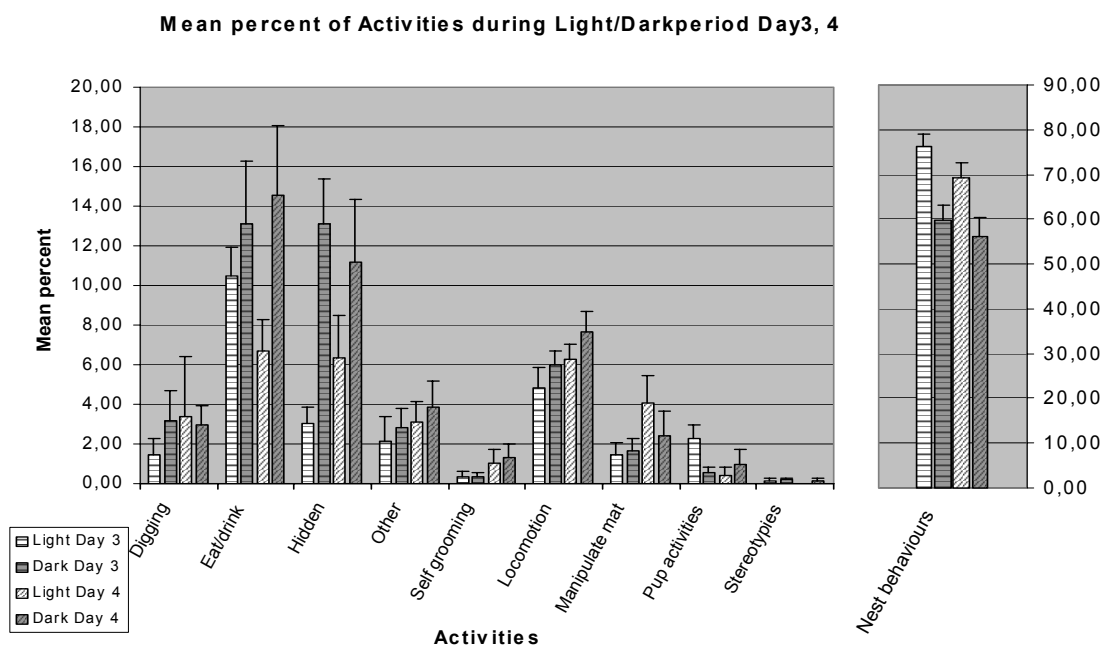


Figure 5. Mean percent (+ S.E.) of Activities during Light/Dark period Day three and four.

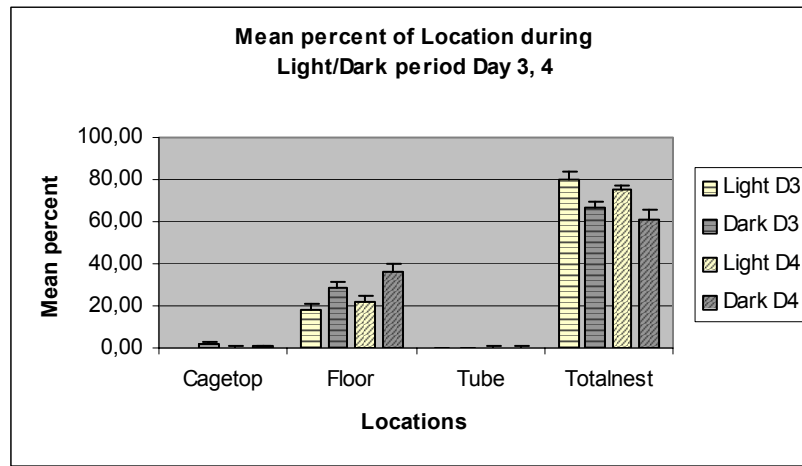


Figure 6. Mean percent (+ S.E.) of Location during Light/Dark period Day three and four.

Pup retrieval test

The female in the barren housing did not complete the retrieval within 15 minutes. All females in the furnished housing retrieved the pups within 5 minutes (see Table 6). The total time for retrieving all pups ranged from 54 seconds to 3 min and 35 seconds, depending on litter size. The mean time for retrieving each pup ranged from 15 seconds to 30 seconds, with a total average of 22 seconds.

Discussion

The aim of this study was to observe maternal behaviour in the inbred strain C57BL/6J, housed in furnished cages. First the behaviour before and after parturition was observed. Females spent significantly more time in the nest after parturition and significantly less time eating/drinking and on locomotory activity. When comparing the behaviour before and after the pup retrieval test, no significant differences in activity or location were found. Neither were there any significant differences between the dark and light period.

The finding that the females spend more time in the nest after parturition is not a surprising result, since newborn pups are fully dependent on their mother for nutrition (König & Markl, 1987). Young from altricial species, such as the house mice, are not capable of maintaining body temperature and are dependent on their mother for warmth. During the first day, the mother usually spends most of the time curled around the pups in the nest (Ewer, 1968). The following two weeks after parturition, females still spend most of their time with the young (König & Markl, 1987; see also Ostermeyer, 1981), declining with increasing pup age (Ewer, 1968; Ostermeyer, 1981).

The present study showed that females do retrieve their pups when the litter is placed outside the nest, as is described also in other studies (see *e.g.* Hennessy *et al.*, 1980; Cohen-Salmon *et al.*, 1985; Pardon *et al.*, 2000). It should be noted that the female from the barren housing behaved differently from the furnished housed females. The female from the barren housing ran around in the cage, digged in the sawdust, and seemed not to pay much attention to the pups. The female failed to retrieve the pups before the maximum time of 15 min (all the females from the furnished housing completed the retrieval within five minutes). The difference in behaviour might be a consequence of the different housing condition. Studies have shown that restrictive housing can cause stress (Olsson & Dahlborn, 2002). However, only one female from barren housing was used in the present study. The difference in behaviour between barren and furnished housed females could therefore also be a consequence of individual differences, not housing conditions.

Separating the pups from the female may be stressful for both the dam and the litter. The behaviour before and after this disturbance was observed to detect any effects of this potential stressor. However, when measured over a period of 12 hours, no alterations in behaviour were found. One reason for this might be that the females were observed for a rather long period after the disturbance, which may have masked a short-term effect of handling. In a study conducted by Priestnall (1973), the effects of handling the litter were observed. Priestnall found an increase in licking offspring during the first hours after the litter had been handled. If the females in the present study had been observed for a shorter period after the pup retrieval test, alterations in behaviour might have been found.

Since mice are nocturnal one could expect a more active behavioural repertoire during the dark period. However, during lactation female mice change their behaviour and during the first days after parturition they spend most of their time in the nest. When comparing the behaviours during dark- and light period no significant differences were found.

This result contradicts the findings made by Wright & Brown (2000). They found more parental behaviours during light than during dark phase. The behaviours they combined to parental behaviours were crouch, rest/touch pups, nest build, lick pup, sniff pup and carry pup. Wright and Brown, however, only observed the females for 10 min/day, for a total of 20 days. This is a short observation time compared to the observation time in the present study (four hour light and eight hour dark period). The difference in observation time could be one reason for the differing results. Another reason for the differing results might be the difference in observation duration. It seems that pooling results measured over a period of 20 days may not give a correct representation, since the behavioural repertoire between the female and the pups changes dramatically over that period: increasing the time the female spends away from the nest (Ewer, 1968; Ostermeyer, 1981) and decreasing the time spent with the pups (König & Markl, 1987).

During the present study a number of females were observed to be infanticidal and cannibalistic. The reason for this behaviour is not fully known. There has been a lot of research concerning the ecological approach to infanticidal behaviour (for a review paper see Labov *et al.*, 1985). According to Hrdy's sexual selection hypothesis, males can benefit from killing non-related pups since this will make the female return to estrus, and thus give the male a sooner opportunity for mating (Labov *et al.*, 1985).

Female mice are reported to kill unrelated pups if the sources in the environment are scarce. There is also experimental evidence of females reducing litter size when food is restricted (Elwood, 1991; König, 1989). McCarthy & vom Saal (1985) reported the majority of virgin and pregnant female mice to be infanticidal towards unrelated young, but virtually no females were infanticidal towards their own offspring at parturition. The release of oxytocin at parturition and during suckling was suggested to impact on the change from infanticidal behaviour to maternal care after parturition in female mice (McCarthy, 1990). Oxytocin is released from vaginal stimulation and suckling by young.

In this study there was one occasion in which the behaviour of an infanticidal female could be observed. The female spent a lot of time away from the young, climbing the cage bars and running around in the cage. Since oxytocin is released during suckling, there might there be a relationship between the amount of time the mothers spent on nursing and infanticidal behaviour. However, no published reports to confirm this relationship was found.

How filming is best carried out

Besides describing maternal behaviour, this study aimed to explore the best possible way of providing cage furniture while maintaining the possibility to carry out behaviour observations. Observing maternal behaviour in furnished cages was a difficult task. All females built complex nests and most often the pups were never seen. All loose objects in the cage were

collected and integrated as a part of the nest. The cardboard tube was often gnawed apart to smaller pieces or used as a nest entrance. This made the behavioural observations difficult. It was often impossible to tell the difference between self-grooming, licking, lifting or moving pups, or manipulating material. It was also difficult to see if the female was nursing or sleeping, since small movements were difficult to detect.

The observed females were filmed from one of four different views (see Table 2). The different views had different advantages. The close view of part of the cage enhanced the chances of detecting when females interacted with the pups. The disadvantage of this view was that nothing but the nest was seen. Therefore it was impossible to observe activities outside the nest. This was also often the case when the cage was filmed from front short side; the complex nest structures blocked the view (see Appendix 1, picture B). When the front long side was in view the females' activities outside the nest was most easy to observe (see Appendix 2, picture C). However, it was difficult to see details in the nest.

When filmed from above the females were provided with a cardboard nest box instead of the MouseHouse. The reason for this was that the females never used the MouseHouse as a nest. This made it impossible to predetermine where to place the cameras. Providing the females with a cardboard nest box made it possible to predetermine where the female would give birth. To film from above was also the only view that made it possible to observe the female inside the nest. When filmed from this view only part of the cage was in view (see Appendix 2, picture D). The advantage with this was that it made a rather close view of the nest box possible, at the same time as most of the females' activities outside the nest could be observed. The disadvantage is that when the camera is placed above, a small part of the cage is hidden below the feed. This problem can be overcome by placing an additional camera in front of the cage. Another disadvantage with this view was that the females sometimes blocked the top of the nest box with material, and made it impossible to observe behaviours inside the nest (see Appendix 3, picture E). Nest material that can be placed on top of the nest should therefore be avoided when filmed from above.

When observing maternal behaviour in furnished cages, one must choose to film from a view that gives the best possibility to observe the behaviours of current interest. Since all views had different advantages it is important to decide which behaviours that are of most interest.

Acknowledgements

I wish to thank Dr. Anna Olsson for giving me the opportunity to come to Portugal to do my honours thesis, and for supervising me during this project. I wish to thank Joana Marques for practical help, and Liliana Silva for taking very good care of the animals used in this study. I also wish to thank Prof. Linda Keeling for functioning as my co-supervisor in Sweden. Finally, I wish to thank Dr. Anna Lundberg for functioning as co-supervisor and Dr. Maria Andersson for functioning as examiner, both with very short notice when I presented this work in Skara.

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APPENDIX 1



A. Cardboard nest box with the top cut of (photo: Robert Eriksson).



B. Front short side.

APPENDIX 2



C. Front long side.



D. From above.

APPENDIX 3



E. From above, with paper tube blocking the view.