uOttawa

L'Université canadienne
Canada's university

FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTOCTORALES

uOttawa

L'Université canadienne
Canada's university

FACULTY OF GRADUATE AND POSDOCTORAL STUDIES

Jessica Sparling
AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.A. (Psychology)
GRADE / DEGREE

School of Psychology
FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

The Consequences of Gestational and Postpartum Environmental Enrichment on Behaviour in the Mother Rat

TITRE DE LA THÈSE / TITLE OF THESIS

Cate Bielajew
DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

Anne Konkle

Méline Plamondon

Gary W. Slater
Le Doyen de la Faculté des études supérieures et postdoctorales / Dean of the Faculty of Graduate and Postdoctoral Studies

Running head: GESTATIONAL AND POSTPARTUM ENVIORNMENTAL ENRICHMENT

The consequences of gestational and postpartum environmental enrichment on behaviour in the mother rat

Jessica E Sparling

May 2010

School of Psychology

University of Ottawa

Your file Votre référence
ISBN: 978-0-494-79678-8
Our file Notre référence
ISBN: 978-0-494-79678-8

Acknowledgements

I would like to acknowledge and offer my sincerest thanks and appreciation to my supervisor, Dr. Catherine Bielajew, for her longstanding involvement in my academic career. Her constant guidance, unwavering support and expert instruction have allowed me to develop valuable research skills and knowledge as well as practice professionalism and responsibility as a graduate student of the University of Ottawa. I would also like to thank my colleagues, Stephanie Baker and Megan Mahoney, for their valuable contributions to this project.

Table of Contents

Acknowledgments 2

Table of Contents 3

Abstract 4

Introduction 6

Methods 11
- Animals 11
- Apparatus 11
- General Procedure 12
- Measures 13
- Statistical Analyses 15

Results 15
- Weight 15
- Litter Characteristics 16
- Elevated-plus maze 16
- Morris water maze 18

Discussion 19

References 25

Table 1 34
Table 2 35

Figure Captions 36

Figures 1 39
Figure 2 40
Figure 3 41
Figure 4 42
Figure 5 43
Figure 6 44
Figure 7 45
Figure 8 46
Figure 9 47

Abstract

Environmental enrichment is known to influence an animal's well-being, provide opportunities for activity, and encourage behaviours appropriate to the species. Female Long-Evans rats were co-housed during their gestational and postpartum times in a colony housing environment comprising numerous cages, with interconnecting tubes, surrounding a multileveled enclosure with many objects scattered throughout. A control group of rats were housed in standard cages. The effects of the physical and social enrichment were determined by evaluating group differences in body weight, litter characteristics, elevated-plus maze performance during the gestational and postpartum periods, and Morris water maze behaviour (postpartum only). Results showed that enriched females were leaner and maintained a constant postpartum weight. Group differences in litter characteristics were observed, with enriched females having heavier but fewer offspring. Behavioural trends were observed in the elevated-plus maze with enriched rats showing greater change in behaviours over time. In the Morris water maze probe test, enriched rats performed less thigmotaxic and more middle maze swimming, as well as an increased tendency to enter the quadrant where the platform was located in non-probe trials. A housing environment, with complex physical and social stimulation, offered more opportunity for environmental interactions producing heartier pups and leaner mothers that displayed differential behavioural responses compared to control mothers. Studying maternal-offspring interactions in a more naturalistic environment allows one to observe a greater repertoire of behaviours that accommodates adequate normal or natural cognitive development than can be observed in the typical standard laboratory housing that limits experience and environmental engagement.

Published as Sparling, Manhoney, Baker, & Bielajew, *The effects of gestational and postpartum environmental enrichment on the mother rat: a preliminary investigation,* in Behavioural Brain Research, 2010, 208, p.213-223.

The consequences of gestational and postpartum environmental enrichment on behaviour of the mother rat

The use of environmental enrichment paradigms has provided a wealth of understanding about how the environment can influence animal cognitive functioning and emotional well-being. Studied since the 1940s, environmental enrichment can be broadly defined as a favourable increase in environmental stimulation thought to provide beneficial experience-dependent physiological changes, promoting behaviours appropriate to the species (Brillaud, Morillion, & de Seze, 2005; Laviola et al., 2004; Elliott & Grunberg, 2005).

Key components of enrichment stimulation are thought to be complexity and novelty (Nithianantharajah & Hannan, 2006; Van Praag, Kempermann, & Gage, 2000). Such stimulation can be provided through physical or social enrichment, which offer different types of environmental engagement (Brillaud, Morillion, & de Seze, 2005; Elliott & Grunberg, 2005; Schrijver, Bahr, Weiss, & Würbel, 2002). Physical enrichment encourages complex cognitive and sensory stimulation through interaction with differently shaped, textured, and coloured objects. It also provides the opportunity for motor and locomotor activity via spatial exploration and navigation. Movement can be facilitated by tunnels, ropes, platforms, running wheels and other objects that may be part of an enriched environment. Likewise engaging, social enrichment activates brain circuits by providing the means for interaction with social conspecifics (Branchi & Alleva, 2006), and thus the development of interactive behaviours.

Overall, enrichment at different stages of growth can induce significant and long-term behavioural and physiological changes in rodents (Larsson, Winblad, & Mohammed, 2002; Chapillon, Patin, Roy, Vincent, & Caston, 2002; Van Praag, Kempermann, & Gage, 2000). Perhaps the most prevalent behavioural finding is that environmental enrichment positively

influences spatial learning and memory in the Morris water maze test (Pham, Söderström, Winblad, & Mohammed, 1999; Falkenberg, et al., 1992; Larsson, Winblad, & Mohammed, 2002; Frick & Fernandez, 2003; Frick, Stearns, Pan, & Berger-Sweeney, 2003; Jankowsky, et al., 2005; Leggio, et al., 2005; Schrijver, Bahr, Weiss, & Würbel, 2002, Mohammed, Winblad, Ebendal, & Lärkfors, 1990; Whishaw, Sutherland, Kolb, & Becker, 1986). Only a few investigators have failed to observe water maze performance differences as a result of enrichment (D'Andrea, Alleva, & Branchi, 2007) with most reporting shorter latencies to locate the hidden platform in enriched animals.

The environment can also affect exploratory behaviour yielding increased inaugural rearing and other exploratory routines (Roy, Belzung, Delarue, & Chapillon, 2001; Escorihuela, Tobeña, & Fernández-Teruel, 1994; Falkenberg, et al., 1992, Brillaud, Morillion, & de Seze, 2005; Larsson, Winblad, & Mohammed, 2002; Mohammed, Winblad, Ebendal, Lärkfors, 1990). In addition, it has been revealed that enriched rats have a more expansive and complex repertoire of exploratory behaviour compared to non-enriched rats, which may foster preparedness to meet environmental challenges and solve problems (Renner, 1987; Renner & Rosenzweig, 1986).

It has also been suggested that enrichment results in superior emotional adaptation and stability as demonstrated by the tendency for enriched animals to show greater initial exploratory response followed by a shorter habituation latency (Pham, et al., 1999; Mohammed, Winblad, Ebendal, & Lärkfors, 1990; Larsson, Winblad, & Mohammed, 2002). Prolonged habituation or high activity in novel situations reflects impaired information-processing and situational anxiety, which in turn may negatively impact an animal's ability to adapt and face environmental changes and challenges (Elliott & Grunberg, 2005). For example, non-enriched animals show more long-term locomotion in novel cages (Falkenberg, et al., 1992), and more overall or prolonged activity in the open field (Brenes, Padilla, & Fornaguera, 2009; Larsson, Winblad, & Mohammed, 2002;

Laviola et al., 2004; Elliott & Grunberg, 2005; Schrijver, Bahr, Weiss, & Würbel, 2002) compared to their enriched counterparts. Other studies show a pattern of behaviour such that enriched animals initially display more behaviours in a novel situation but decrease or cease exploring sooner than non-enriched animals (Mohammed, Winblad, Ebendal, & Lärkfors, 1990; Larsson, Winblad, & Mohammed, 2002). In the open field test, enriched animals show higher rearing scores compared to non-enriched animals in the first 10 min and then lower rearing scores in the remainder of the test (Mohammed, Winblad, Ebendal, & Lärkfors, 1990; Larsson, Winblad, & Mohammed, 2002). Also in the open field test, enriched rats showed more movement in the central area and less thigmotaxis followed by quicker habituation (Brenes, Padilla, & Fornaguera, 2009). Learning can result from natural exploration of objects; however exploration also has the potential to make the animal vulnerable (Renner & Rosenzweig, 1986) to predators or other environmental threats. Presumably, efficiency in gaining knowledge through exploration has had an evolutionary advantage (Renner & Rosenzweig, 1986).

Conflictingly, enrichment has been shown to both decrease (Benaroya-Milshtein, et al., 2004; Friske & Gammie, 2005; Peña, Prunell, Dimitsantos, Nadal, & Escorihuela, 2006) and increase (Branchi & Alleva, 2006; Zhu, et al., 2006) behaviour in the open arms of the elevated-plus maze, interpreted to reflect higher and lower anxiety respectively. The effect of environmental enrichment on the HPA axis is likewise inconsistent with some studies reporting decreased basal corticosterone concentrations (Belz, Kennell, Czambel, Rubin, & Rhodes, 2003) and others, increased basal corticosterone concentrations (Martínez-Cué, et al., 2005; Marashi, Barnekow, Ossendorf, & Sachser, 2003; Benaroya-Milshtein, et al., 2004; Moncek, Duncko, Johansson, & Jezova, 2004). At the same time, environmental enrichment has been associated with decreases in the HPA axis response to stressor (Roy, Belzung, Delarue, & Chapillon, 2001; Belz, Kennell, Czambel, Rubin, & Rhodes, 2003; Benaroya-Milshtein, et al., 2004) while others

report no group differences between enriched and control animals in this context (Falkenberg, et al., 1992; Moncek, Duncko, Johansson, & Jezova, 2004; Larsson, Winblad, & Mohammed, 2002). Furthermore, investigators have shown that both enriched and non-enriched animals show a corticosterone response to the first exposure of a handling stress, yet additional exposure to the same stress produces a corticosterone response only in the non-enriched group (Moncek, Duncko, Johansson, & Jezova, 2004). This pattern of responding is similar to the finding that enriched animals show quicker habituation to novel stimuli than control animals, suggesting that enrichment encourages timely adaptation to environmental demands.

A number of studies have examined the effects of gestational environmental stimulation on subsequent offspring cognitive and emotional development (Branchi & Alleva, 2006; Branchi, 2008; Welberg, Thrivikraman, & Plotsky, 2006). Although these studies address a worthwhile topic, it should also be recognized that the gestational and postpartum periods are times of growth for the mother. The maternal animal is dynamic in nature, with interconnected changes to her brain circuitry, behaviour, and hormone levels (Kinsley & Lambert, 2008). In fact, the maternal experience has been described by some as being akin to environmental enrichment, resulting in positive behavioural changes as compared to virgin counterparts (Kinsley & Lambert, 2008). Moreover, attenuation of HPA axis responsiveness occurs naturally during the gestational and postpartum periods (Neumann, et al., 1998; Douglas, Brunton, Bosch, Russell, & Newmann, 2003). It is unknown how an environmental enrichment protocol impacts the female during these sensitive phases.

In animal models of gestational stress, restraint stress has been show to have lasting effects on the dam rat, resulting in increased behavioural anxiety in the elevated-plus maze (Baker, et al., 2008; Darnaudéry, Dutriez, Viltart, Morley-Fletcher, & Maccari, 2004). The same result is not observed in restrained virgin rats, suggesting that the gestational period is a time in

which females are particularly vulnerable (Darnaudéry, Dutriez, Viltart, Morley-Fletcher, & Maccari, 2004).

With this background, the present study evaluated the effects of an enriched housing environment on the mother rat. A large enrichment cage, called the social colony, provided a common home for enriched female rats throughout their gestational and postpartum periods. The social colony comprised a network of cages equipped with a variety of objects, platforms, tunnels, ropes, and spacious areas to explore - all providing physical enrichment. Co-housed in the social colony apparatus, the enriched dams and their litters lived in an interactive community offering social enrichment. In addition, the social colony made available communal areas for nesting, allowing the enriched families' to nest according to their natural tendency (D'Andrea, Alleva, & Branchi, 2007; Branchi & Alleva, 2006; Hayes, 2000). We assessed the effects of this enriching environment on body weight, litter characteristics, and performance in the Morris water maze and elevated-plus maze and compared our findings to mother rats and their litters housed in standard laboratory conditions.

The present study is unique in two ways: (1) the social colony is an elaborate and comprehensive enrichment condition, with only few studies employing comparable apparatuses (Brenes Sáenz, Villagra, & Fornaguera, 2006; Polley, Kvasnák, & Frostig, 2004; Roy, Belzung, Delarue, & Chapillon, 2001); (2) the focus of this study is the effect of gestational and postpartum enrichment on the mother rat rather than her offspring.

Methods

Animals

Ten virgin female Long-Evans rats (age 45-55 days) from Charles River Laboratories, Quebec, Canada, were used in the present study. Upon arrival, they were housed two per cage for a 20 day period of acclimatization to their new environment. The cages were constructed of transparent polycarbonate (24 cm x 43 cm x 20 cm), with metal lids, containing sawdust bedding and one plastic tube per cage. Food pellets (Harane Rodent Diet, 8012) and tap water were available ad libitum. The room temperature was maintained at 23 ± 2°C with relative humidity levels of 40 ± 5%; lights were on at 07:00 hr and off at 19:00 hr. At 65-75 days of age, the rats were randomly assigned to one of two housing conditions, either a control (n=5) condition, housed singly in standard-sized cages (same dimensions and description as above), or an experimental (n=5) social colony housing condition (see dimensions below).

Social Colony Housing Apparatus

The social colony was designed to provide physical and social stimulation. It consisted of six standard-size rat cages surrounding a larger custom made four-level middle cage (52 cm x 43 cm x 77.5 cm); all standard cages were connected to adjacent standard cages and the larger middle cage by tubes (6.35 cm in diameter) to allow movement around the colony; Figure 1 shows a photograph of the apparatus. A wide variety of toys of different colours and textures were dispersed throughout the structure to provide further physical stimulation. These included plastic chew toys, plastic chain links, climbing ropes, toy bells, nesting materials, small plastic balls, and tubes. The toys were replaced regularly with similar items to maintain novelty, as is common in enrichment paradigms (Bevins & Besheer, 2005; Moncek, Duncko, Johansson, & Jezova, 2004; Nithianantharajah & Hannan, 2006).

General Procedure

All rats were bred approximately nine days after assignment to their housing condition. Figure 1 shows a timeline of events from the breeding phase until the end of the study. Rats remained in their assigned housing condition (social colony or control) from breeding until weaning, approximately 26 days after parturition. Receptive rats were chosen for breeding, based on proestrous or estrous readings of vaginal smears, were paired with a sexually experienced male Long-Evans rat for three consecutive 24 hr periods. During breeding, each socially housed rat and her mating partner were isolated in one of the social colony cages by blocking the interconnecting tubes to prevent movement in the colony during the breeding phase. Conception was identified by the presence of sperm in a vaginal smear and confirmed by weight gain over time. Control rats were bred using identical procedures. Body weights were recorded daily until offspring were weaned.

From gestation day (GD) 20 until post-natal day (PND) 7, rats from the social colony were isolated in their cages, again to prevent exploration of the colony. During this period, the cages were left undisturbed and contained no tubes or toys. To promote nest building, pregnant rats were given a few Nestlets® each day. The day of parturition was considered PND 1.

On PND 2, the number of offspring per dam was counted, sexed, weighed, and culled to ten pups per dam, five of each sex if possible. Each litter was culled to ten pups; two deaths occurred after the culling procedure, thus resulting in the following number of offspring per condition: control female offspring (25), control male offspring (25), colony female offspring (23), and colony male offspring (25). On PND 7, control dams and offspring were placed in large cages (48 × 37 × 20 cm), one family per cage; no tubes were supplied to these cages. At the same time, the tubes connecting the cages in the social colony were unblocked.

All colony litters were born within four days of each other and all control litters were born within six days of each other. Since the colony litters were co-housed in the social colony, testing and weaning occurred concurrently for all rats. For the remainder of the manuscript the notion APND, meaning average postnatal day will denote concurrent procedural events.

Pre-weaning procedures were adapted from Cook (1999) in order to decrease the stress associated with the process. Six days prior to weaning, dams were removed from their home cage for increasing amounts of time (Cook, 1999). The dams were removed for 3 hr, 3 hr, 6 hr, 6 hr, 9 hr, and 9 hr per day in that order and then weaned completely on the seventh day (APND 26). During pre-weaning isolation and once weaned, colony mothers were co-housed in a large cage (48 × 37 × 20 cm) with inanimate enrichment provided through tubes, Nestlets® and other toys. Control mother were housed individually in standard sized cages.

Measures

All measures were obtained during the light phase, between 09:00 hr and 17:00 hr recorded by a digital camcorder (JVC GZ-MG30U); the data were scored using Observational Data Logging (OD Log) software.

Weight & Litter Characteristics

Daily weights of the female rats prior to breeding, throughout gestation, and during postpartum were measured. Prior to culling on PND 2, the litter characteristics (total litter weight and number of offspring) for each dam were recorded.

Elevated plus maze

The elevated plus maze apparatus comprised four arms made of grey painted plywood, extending from a central square (14 cm). One set of opposing arms (38cm × 14cm) was enclosed by 30 cm high walls (closed arms) and the other set remained open (open arms). The maze was elevated 32 cm above the floor. All tests were preceded by at least 30 min of acclimatization in

the test room. Each individual session lasted 15 min. The apparatus was cleaned with 70% ethanol between tests.

To begin each test, rats were placed in the center of the apparatus facing an open arm. Arm entry was defined as the presence of the hind paws in the arm. Rats were tested on GD 19, and then again on APND 27. For each arm of the maze, duration, frequency as well as the transformed variable of duration per frequency were used as dependent measures. In addition, frequency and duration of rearing, grooming, and edge behaviour were assessed.

Morris Water Maze

A large oval pool was used as a water maze (length: 174 cm; width: 127 cm; height: 40 cm). The maze was filled with room temperature water (height: 21 cm) made opaque by the addition of non-toxic tempera paint. Stationary objects surrounding the maze were not hidden from sight and therefore acted as immobile visual cues. The water maze was divided into equal quadrants. A cylindrical platform (diameter: 11 cm, height: 20 cm) was submerged in the maze 1 cm beneath the water surface in the middle of one of the quadrants, approximately 38 cm from the pool wall.

The procedure consisted of four blocks of four trials. Two blocks of trials were conducted per day for two consecutive days, with a probe test at the end of the second day. In the trials, there was a platform submerged just below the water surface, while in the probe test the platform was removed. The testing was conducted on APND 38 and 39. For each non-probe trial, rats were placed into the MWM facing the wall at one of the quadrant boundaries. The point of entry was counterbalanced for condition and subject with each subject placed at a different boundary once per block of four trials. Each rat was left in the water maze for a maximum duration of 2 min or until the platform was found. The platform was always positioned in the same quadrant. Latency to locate the platform was recorded. If the rat did not find the platform, it was guided to

and placed on it. Failure to find the platform was observed rarely and only on the first trial. Once the rat reached the platform it was left there for 10 s; during this brief period, no rat was observed to leave the platform.

For the probe test, rats were left in the pool for 2 min. Coordinate of entry for the probe trial was counterbalanced for condition. In the probe test, the frequency of entry into the quadrant that had contained the platform and the duration of time in it were recorded as well as the transformed score of duration per entry. Finally, the frequency of swimming in the vicinity of the former platform location, the frequency of swimming in the middle of the maze, and the amount of time spent swimming at the periphery of the maze were recorded.

Statistical Analyses

All analyses were performed using the statistical package SPSS 17.0; omnibus tests and planned comparisons were evaluated at an alpha level of 0.05 and unplanned post-hoc comparisons using a corrected Bonferroni alpha level. Mixed ANOVAs with the Huynh-Feldt correction for sphericity [64] were used to analyse the weight, EPM, and MWM trial data; any deviations from sphericity results in an adjustment to the degrees of freedom. Group differences in the MWM probe tests were evaluated using one-way ANOVAs. Non-parametric tests were applied to the evaluation of litter number.

Follow-up tests on the EPM data were conducted using linear regression analyses to determine group differences in slope. Due to the number of variables examined, the alpha level was set at 0.004 to correct for multiplicity.

Results

Weight

The comparison of pre-housing weight showed no significant difference between groups (colony = 281 ± 6.5g and control = 280 ± 7.1g). Weight data through the gestation (GD 1-22)

and postpartum periods until weaning (PND 2-26) are plotted in Figure 2. There was a significant main effect of day ($F(8.231, 65.845) = 112.459$, $p < 0.001$) and interaction between day and housing group ($F(8.231, 65.845) = 4.207$, $p < 0.001$). Further post-hoc analyses via a mixed ANOVA on the postpartum data produced a significant main effect of day ($F(9.648, 77.186) = 13.005$, $p < 0.001$) and an interaction, with change in weight over time depending on housing condition, ($F(9.648, 77.186) = 5.424$, $p < 0.001$). The colony dams maintained stable postpartum weight ($F(7.948, 31.792) = 1.336$, $p = 0.262$) while control dams showed significant change in weight over the same period ($F(12.820, 51.281) = 22.594$, $p < 0.001$). Supplementary selected between-group comparisons were conducted on days PD12 through 22. No differences were found based on a Bonferroni corrected alpha level of $p = 0.001$.

Litter Characteristics

Figure 3 shows between-group comparisons of pre-culling litter weight, pup number, and individual pup weight. Total litter weight comparisons yielded no significant difference between housing condition ($F(1, 8) = 0.687$, $p = 0.431$). On the other hand, a Mann-Whitney U comparison of the number of pups per litter revealed that control dams had significantly larger litters ($U = 2$, $p = 0.032$). In addition, average pup weight per litter was shown to significantly differ between housing groups ($F(1, 8) = 22.446$, $p = 0.001$); colony dams were associated with heavier pups (6.90 ± 0.1 g) compared to control dams (5.99 ± 0.1 g), an overall average 15% difference.

Elevated-Plus Maze

Given the number of variables examined in the EPM analyses (duration, frequency, and duration per entry for open and closed arms and duration and frequency for rearing, grooming, and edge behaviour; total = 12), we chose to list all significant results associated with the

omnibus tests in Tables 1 and 2. The gestational EPM data are shown in Figures 4 and 5 and the postpartum EPM data in Figures 6 and 7.

Gestational data

Refer to Table 1 and Figures 4 and 5. All significant main effects of interval were associated with a decrease over time, except for total grooming frequency which increased across intervals.

Housing comparisons revealed that colony dams groomed significantly more often than control dams (Figure 5E).

No significant interactions between housing condition and interval were found.

Further analyses were conducted to evaluate the slopes of the individual groups over the three intervals. Of the 12 linear regression analyses applied to these data, significant slope differences were found in seven cases – closed arm duration and duration per entry, open arm duration per entry, and duration and frequency of grooming and frequency of rearing and edge behaviour. The p values associated with these analyses appear in the right top corner of each plot of Figures 4 and 5. Significant findings were based on a Bonferroni-corrected alpha level $\leq$ 0.004.

Based on the results of the transformed variable, duration per entry, the overall pattern of closed and open arm behaviour indicates that the colony group increased the time spent over time per entry in both closed and open arm portions of the maze relative to that of the control group. Similarly, the colony rats tended to groom more frequently and for longer periods, and display rearing and edge behaviour less often than their reference group.

Postpartum data

Refer to Table 1 and Figures 6 and 7. Significant main effects of interval were found for all duration and frequency data associated with closed arm, open arm, rearing and edge variables.

Main effects of housing group were observed for rearing frequency and grooming duration; colony rats displaying a lower frequency of rearing and a higher amount of grooming behaviour than that observed in control rats. Finally, an interaction between group and interval was obtained for the transformed variable, duration per entry, in the case of the open arm.

The results of the linear trend analyses resulted in significant slope differences in all variables except for open arm duration per entry, grooming frequency and edge duration.

The pattern emerging from the postpartum evaluation on EPM indicates that overall colony rats spent significantly more time per entry in the closed arm than was observed in the control rats (slope values: colony = 19.4, control = 7.6). No group differences were found in open arm behaviour for the same variable, the slopes being almost identical. Colony rats also reared less and for shorter periods (see Figure 7A & D) and groomed for longer periods (see Figure 7B).

Gestational vs. Postpartum data

Finally the results of the EPM tests conducted during the gestational and postpartum periods were compared using a 2x2x3 mixed ANOVA, with housing group, test period (gestation vs postpartum) and interval as independent variables. The significant results appear in Table 2. No three-way interactions were found. Main effects of interval on frequency of behaviour were observed in several variables. Housing differences were obtained in closed arm and grooming frequency. As well, a difference between performance in the gestational versus postpartum tests was found for edge duration with more edge behaviour postpartum. The two-way interaction between interval and test period was significant; however, since the term did not include the housing factor, it was not further investigated. Thus, the results suggest that both gestational and postpartum EPM tests produced similar performance in each housing group.

Morris Water Maze Trials

Latency data related to the MWM are shown in Figure 8. Overall, there was a main effect of trial ($F(7.417, 59.337) = 19.652$, $p < 0.001$) due to a significant decrease in latency in both groups to reach the platform. No other significant differences were found.

Morris Water Maze Probe

Figure 9 shows the results of the probe test on measures of duration, frequency, and duration per entry related to the quadrant containing the platform in the trial test (left column). Recall that the platform is removed in the probe test. The data for other measures including the frequency to approach the platform position, the frequency of middle maze swimming, and the duration of thigmotactic or periphery swimming are located in the right column of the figure.

Group differences were found in the length of time per entry to the platform quadrant ($F(1, 8) = 7.739$, $p = 0.024$), frequency of entry to platform quadrant ($F(1, 8) = 7.714$, $p = 0.024$), frequency of swimming in the center of the maze ($F(1, 8) = 9.191$, $p = 0.016$), and thigmotactic swimming ($F(1, 8) = 9.091$, $p = 0.017$).

While control rats spent significantly more time per entry in the platform quadrant, the colony rats frequented it more. Colony rats were more likely to swim in the center of the maze while control rats spent more time in the periphery of the maze.

Discussion

This investigation provides preliminary findings on the impact of environmental factors during the gestational and postpartum periods. Enrichment in the form of physical and social interaction has a positive influence on the physical health of the mother and offspring. Weight is often regarded as a reflection of animal well-being (Brillaud, Morillion, & de Seze, 2005), and although there was no influence of enrichment on weight gain during gestation, the enrichment paradigm produced leaner mothers that maintained a constant postpartum weight. This is consistent with most investigations of environmental enrichment showing that enriched

animals show comparable leaner (Moncek, Duncko, Johansson, & Jezova, 2004; Olsson & Dahlborn, 2002; Larsson, Winblad, & Mohammed, 2002; Brillaud, Morillion, & de Seze, 2005; Pham, Söderström, Winblad, & Mohammed, 1999) and more stable weight over time (Brillaud, Morillion, & de Seze, 2005).

The weight of the offspring at birth is likewise affected with social colony dams birthing heartier pups. In mice, it has been reported that physically enriched mothers produced smaller litters, yet bear heavier pups (Tsai, Oppermann, Stelzer, Mähler, & Hackbarth, 2003). Plumper offspring is often referenced as a predictor of developmental success (Byrd & Weitzman, 1994; Prathanee, et al., 2009), suggesting that the colony pups would have a physiological advantage compared to their non-enriched mates.

Validated for assessment of anxiety-like behaviour (Pellow, Chopin, File, & Briley, 1985), the EPM test traditionally focuses on the behaviour in the open arm. Our EPM test results suggest no differences in anxiety-like behaviour, based on failure to detect group differences in the open arm. Moreover, as suggested by Montgomery (1955), the initial 5 minutes of maze exposure shows the greatest amount of avoidance behaviour for open maze arms (Montgomery, 1955). Although not reported in our results section, we did analyze the first 5 minutes of each EPM test in a minute-by-minute fashion and found no significant group differences for any open or closed arm measures. Analysis of the 15 minutes test permitted identification of differences in group exploratory behaviour over time.

Interestingly though, a striking pattern was observed from analysis of behavioural trends in the EPM via linear regression. In these trends, enriched mothers showed a steeper slope or greater changes in behaviour over time. For example, enriched mothers showed increased duration per entry in the maze arms indicating decreased locomotion between arms. More prominent decreases in arm, edge, and rearing frequency meant that enriched females attenuated

activity and exploratory behaviours over time. Enriched females also showed greater increases in grooming and closed arm duration over the course of the test. While individually each of these trends is not particularly significant, taken as a whole, these findings suggest that enriched rats may acclimatize to their environment faster in unfamiliar situations.

A decrease in rearing or locomotor behaviour over time is evidence for habituation to a situation; in addition, increased grooming over time further supports habituation (Brenes, Padilla, & Fornaguera, 2009). As previously mentioned habituation can be a reflection of faster cognitive processing, leading to success in dealing with a novel situation. Activity level has been shown to be related to anxiety, specifically in the open field test, with hyperactivity reflecting a state of higher emotional reactivity (Crawley, 1985; Ramamoorthy, Radhakrishnan, & Borah, 2008; Brenes Sáenz, Villagra, & Fornaguera, 2006). Grooming also is interpreted as representing decreased anxiety (Brenes Sáenz, Villagra, & Fornaguera, 2006), as it requires attention to be redirected from the surroundings to self (Brenes, Padilla, & Fornaguera, 2009). However, as the EPM is not validated to interpret habituation, exploration, and cognitive strategies, we cannot conclude that the colony mother did indeed habituate faster. At the same time, the EPM results find support in the literature. For example, socially enriched mice may habituate quicker to novel situations (Pham, et al., 1999; Mohammed, Winblad, Ebendal, Lärkfors, 1990; Larsson, Winblad, & Mohammed, 2002) including novel EPM exposure, spending more time grooming and showing a behavioural trend towards fewer arm entries (Branchi & Alleva, 2006).

There was no difference in open and closed arm behaviours for housing groups between the gestational and postpartum EPM tests. In test-retest protocols in the EPM, frequently used to test pharmacological agents related to anxiety, the second maze exposure is often associated with increased open arm avoidance (Bevins & Besheer, 2005; Rodgers, Lee, & Sheperd, 1992), thought to reflect a learned fear or avoidance response (Bevins & Besheer, 2005;

Carobrez & Bertogilo, 2005). However, earlier studies have found no support for increased open arm performance with repeated maze exposure (File, Mabbutt, & Hitchcott, 2003; Lister, 1987; Pellow, Chopin, File, & Briley, 1985). It should also be noted that the two EPM tests conducted in this study did not use a typical protocol of 24 or 48 hour test-retest intervals, and that when longer intervals are used (3 weeks), control rats show no change in EPM behaviour (Adamec & Shallow, 2000). In addition to the fact that our gestational and postpartum EPM tests were separated by 31 days, it is also important to recognize that the gestational and postpartum periods correspond to different endocrine and environmental demands, developing from pregnancy to motherhood. Thus, the control group is believed to represent the best test-retest reference from gestation to postpartum in this study. Our only observation was an overall increase in edge behaviour between the gestational and postpartum tests.

On a side note, the behaviour differences in EPM exploration may warrant that future studies of gestational and postpartum enrichment use more accepted measures to assess habituation, emotional reactivity, and cognition like the open field test, t-maze, or the hole-board test. Perhaps comprehensive social and physical enrichment during the gestational and postpartum times prepares subjects to meet environmental demands.

The MWM protocol to locate the hidden platform did not produce any group differences in spatial learning and memory. It is possible that the non-probe task was not sufficiently challenging to distinguish any housing-related differences in spatial learning and navigation. It would be worthwhile to test this finding in future models of gestational enrichment with a more demanding MWM protocol. For example, changing platform location (D'Hooge & Be Beyn, 2001, Van der Borght, et al., 2005; Morris, 1984), or utilizing fewer distal cues (Lamberty & Gower, 1991) may prove more challenging.

The probe test revealed that environmental enrichment reduced thigmotaxic swimming behaviour and increased swimming through the center of the maze. Thigmotaxis, an index of anxiety in rats (Treit & Fundytus, 1988; Simon, Dupuis, Costentin, 1994; Calabrese, 2008), has been shown to be more often displayed by non-enriched rats in open field tests (Branchi & Alleva, 2006; Brenes, Padilla, & Fornaguera, 2009). Swimming though the maze center reflects an adaptive approach to locating the hidden platform in the MWM task (Schulz, Topic, De Souza, & Huston, 2004), while thigmotaxic swimming reflects a maladaptive strategy (Schulz, Topic, De Souza, & Huston, 2004), and is thought to be due to anxiety or fear (Devan & McDonald, 1999). Our enriched rats have much more experience with novelty and more complex physical environments and as a result, had the opportunity to display more physical activity than control rats.

Schulz, Huston and colleagues (2007 & 2008) have shown that a lower resistance to extinction in the MWM is related to increased immobility (a reflection of despair) and postulate that higher resistance to extinction may decrease the likelihood of developing despair or depression (Schulz, Buddenberg, & Huston, 2007; Schulz, Huston, Buddenberg, & Topic, 2007; Topic, et al., 2008). Although we did not asses this phenomenon through extinction trials or other measures of despair, like the forced swim test, the outcome from the probe test suggests that both of these behavioural assessments would be interesting to conduct in future studies. In this way, we could evaluate if gestational and postpartum environmental enrichment have protective effects, decreasing susceptibility to despair, anxiety, and/or depression.

Sharing common elements with natural-type housing apparatuses (Polley, Kvasnák, & Frostig, 2004), the social colony provides a wealth of environmental stimulation. The housing environment of laboratory animals is often limited, with small space and lack of stimulation, devoid of novel or complex objects as well as conspecifics. In standard laboratory housing, so

described as impoverished (Van Praag, Kempermann, & Gage, 2000), animals often have a limited behavioural repertoire as a consequence of the housing confines (Olsson & Dahlborn, 2002). Standard housing conditions are sufficient to meet physiological requirements (Olsson & Dahlborn, 2002), but offer negligible engagement of mental and physical faculties of the animal (Van Waas & Soffie, 1996). Housing can produce significant and long lasting changes to behaviour and physiology, so it is important to provide an environment that accommodates adequate normal or natural cognitive development in order to achieve quality scientific findings. The study of environmental implications of housing is important for animal welfare and interpretation of animal experiments (Schrijver, Bahr, Weiss, & Würbel, 2002). The ideal would be to have a standard housing environment that is both cost effective and approximates the conditions for normal cognitive and behavioural development.

In our study, life in the social colony was more demanding than that offered by the standard housing condition, requiring daily use of spatial navigational strategies, and the ability to deal effectively with novel objects, to address peers, and to participate in social group dynamics. Thus, enriched mothers were more active, placing greater demands on cardiovascular and muscular systems. During the postpartum phase, the dams had the additional requirements of providing maternal care to offspring and overseeing pup locomotion and interaction. This preliminary study shows that both the behaviour and physiology of mothers can be affected in beneficial ways by applying a combination of physical and social enrichment during the dynamic gestational and postpartum phases.

References

Adamec, R., Shallow, T. (2000). Effects of baseline anxiety on response to kindling of the right medial amygdala. *Psysiol Behav, 70,* 67-80.

Baker, S., Chebli, M., Rees, S., Lemarec, N., Godbout, R., Bielajew, C. (2008). Effects of gestational stress: 1. Evaluation of maternal and juvenile offspring behavior. *Brain Res, 1213,* 98-110.

Belz, E., Kennell, J., Czambel, R., Rubin, R., Rhodes, M. (2003). Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. *Pharmacol Biochem Behav, 76,* 481-486.

Benaroya-Milshtein, N., Hollander, N., Apter, A., Kukulansky, T., Raz, N., Wilf, A., Yaniv, I., Pick, C. (2004). Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. *Eur J Neurosci, 20,* 1341-7.

Bertoglio, L., Carobrez, A. (2000). Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus-maze model of anxiety. *Behav Brain Res, 108,* 197-203.

Bevins, R., Besheer, J. (2005). Novelty reward as a measure of anhedonia. *Neurosci Biobehav Rev, 29,* 707-14.

Branchi, I., Alleva, E. (2006). Communal nesting, an early social enrichment, increases the adult anxiety-like response and shapes the role of social context in modulating the emotional behavior. *Behav Brain Res, 172,* 299-306.

Branchi I. (2008). The mouse communal nest: Investigating the epigenetic influences of the early social environment on brain and behavior development. *Neuriscu Biobehav Rev, 33,* 551-9.

Brenes, J., Padilla, M., Fornaguera, J. (2009). A detailed analysis of open-field habituation and

behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behav Brain Res, 197,* 125-37.

Brenes Sáenz, J., Villagra, O., Fornaguera Trías, J. (2006). Factor analysis of Forced Swimming test, Sucrose Preference test and Open Field test on enriched, social and isolated reared rats. *Behav Brain Res, 169,* 57-65.

Brillaud, E., Morillion, D., de Seze, R. (2005). Modest environmental enrichment: effect of a radial maze validation and well being of rats. *Brain Res, 1054,* 174-182.

Byrd, R., Weitzman, M. (1994) Predictors of early grade retention among children in the United States. *Pediatrics, 93,* 481-7.

Calabrese E. (2008). An assessment of anxiolytic drug screening tests: hormetic dose responses predominate. *Crit Rev Toxicol, 38,* 489-542.

Carobrez, A., Bertogilo, L. (2005). Ethological and temporal analyses of anxiety-like behaviour: The elevated-plus maze model 20 years on. *Neurosci Biobehav Rev, 29,* 1193-205.

Chapillon, P., Patin, V., Roy, V., Vincent, A., Caston, J. (2002). Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: a review. *Dev Psychobiol, 41,* 373-87.

D'Andrea, I., Alleva, E., Branchi, I. (2007).Communal nesting, an early social enrichment, affects social competences but not learning and memory abilities at adulthood. *Behav Brain Res, 183,* 60-6.

D'Hooge, R., De Deyn, P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Res Brain Res Rev, 36,* 60-90.

Darnaudéry, M., Dutriez, I., Viltart, O., Morley-Fletcher, S., Maccari, S. Stress during gestation induces lasting effects on emotional reactivity of the dam rat. *Behav Brain Res, 153,* 211-216.

Devan, B., McDonald, R., White, N. (1999). Effects of medial and lateral caudate-putamen lesions on place- and cue-guided behaviors in the water maze: relation to thigmotaxis. *Behav Brain Res, 100,* 5-14.

Douglas, A., Brunton, P., Bosch, O., Russell, J., Newmann, I. (2003). Neuroendocrine responses to stress in mice: hyporesponsiveness in pregnancy and parturition. *Endocrinology, 144,* 5268-76.

Cook, J. (1999). Patterns of weaning and adult response to stress. Physiol Behav, 67, 803-8.

Crawley, J. (1985). Exploratory behavior models of anxiety in mice. *Neurosci Biobehav Rev, 9,* 37-44.

Elliott, B., Grunberg, N. (2005). Effects of social and physical enrichment on open field activity differ in male and female Sprague-Dawley rats. *Behav Brain Res, 165,* 187-96.

Escorihuela, R., Tobeña, A., Fernández-Teruel, A. (1994). Environmental enrichment reverses the detrimental action of early inconsistent stimulation and increases the beneficial effects of postnatal handling on shuttlebox learning in adult rats. *Behav Brain Res, 61,* 169-73.

Falkenberg, T., Mohammed, A., Henriksson, B., Persson, H., Winblad, B., Lindefors, N. (1992). Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment. *Neurosci Lett, 138,* 153-6.

File, S., Mabbutt, P., Hitchcott, P. (1990). Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effects of chlordiazepoxide in the elevated plus-maze. *Psychopharm, 102,* 98-101.

Frick, K., Fernandez, S. (2003). Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice. *Neurobiol Aging, 24,* 615-26.

Frick, K., Stearns, N., Pan, J., Berger-Sweeney J. (2003). Effects of environmental enrichment on spatial memory and neurochemistry in middle-aged mice. *Learn Mem, 10,* 187-98.

Friske, J., Gammie, S. (2005). Environmental enrichment alters plus maze, but not maternal defense performance in mice. *Physiol Behav, 85,* 187-94.

Hayes, L. (2000). To nest communally or not to nest communally: a review of rodent communal nesting and nursing. *Anim Behav, 59,* 677-88.

Jankowsky, J., Melnikova, T., Fadale, D., Xu, G., Slunt, H., Gonzales, V., Younkin, L., Younkin, S., Borchelt, D., Savonenko, A. (2005). Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J Neurosci, 25,* 5217-24.

Kinsley, C., Lambert, K. (2008). Reproduction-induced neuroplasticity: natural behavioural and neuronal alterations associated with the production and care of offspring. *J Neuroendocrinol, 20,* 515-25.

Lamberty, Y., Gower, A. (1991). Simplifying environmental cues in a Morris-type water maze improves place learning in old NMRI mice. *Behav Neural Biol, 56,* 89-100.

Larsson, F., Winblad, B., Mohammed, A. (2002). Psychological stress and environmental adaptation in enriched vs. impoverished housed rats. *Pharmacol Biochem Behav, 73,* 193-207.

Laviola, G., Rea, M., Morley-Fletcher, S., Di Carlo, S., Bacosi, A., De Simone, R., Bertini, M., Pacifici, R.. Beneficial effects of enriched environment on adolescent rats from stressed pregnancies. *Eur J Neurosci, 20,* 1655-64.

Leggio, M., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behav Brain Res, 163,* 78-90.

Lister, R. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology, 92,* 180-5.

Marashi, V., Barnekow, A., Ossendorf, E., Sachser, N. (2003). Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. *Horm Behav, 43,* 281-92.

Martínez-Cué, C., Rueda, N., García, E., Davisson, M., Schmidt, C., Flórez, J. (2005). Behavioral, cognitive and biochemical responses to different environmental conditions in male Ts65Dn mice, a model of Down syndrome. *Behav Brain Res, 163,* 174-85.

Mohammed, A., Winblad, B., Ebendal, T., Lärkfors, L. (1990). Environmental influence on behaviour and nerve growth factor in the brain. *Brain Res, 528,* 62-72.

Moncek, F., Duncko, R., Johansson, B, Jezova, D. (2004). Effect of environmental enrichment on stress related systems in rats. *J Neuroendocrinol, 16,* 423-31.

Montgomery, K. (1955). The relation between fear induced by novel stimulation and exploratory behaviour. *J Comp Physiol Psychol, 48,* 254-60.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods, 11,* 47-60.

Neumann, I., Johnstone, H., Hatzinger, M., Liebsch, G., Shipston, M., Russell, J., Landgraf, R., Douglas, A. (1998). Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohypophysial changes. *J Physiol, 508,* 289-300.

Neumann, I., Krömer, S., Bosch, O. (2005). Effects of psycho-social stress during pregnancy on neuroendocrine and behavioural parameters in lactation depend on the genetically determined stress vulnerability. *Psychoneuroendocrinol, 30,* 791-806.

Nithianantharajah, J., Hannan, A. (2006). Enriched environments, experience-dependent

plasticity and disorders of the nervous system. Nature Reviews Neuroscience, 7, 697-709.

Olsson, A., Dahlborn, K. (2002). Improving housing conditions for laboratory mice: a review of 'environmental enrichment'. *Lab Animals, 36,* 243-270.

Peña, Y., Prunell, M., Dimitsantos, V., Nadal, R., Escorihuela, R. (2006). Environmental enrichment effects in social investigation in rats are gender dependent. *Behav Brain Res, 174,* 181-7.

Pellow, S., Chopin, P., File, S., Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods, 14,* 149-67.

Pham, T., Ickes, B., Albeck, D., Söderström, S., Granholm, A., Mohammed, A. (1999). Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. *Neuroscience, 94,* 279-86.

Pham, T., Söderström, S., Winblad, B., Mohammed, A. (1999). Effects of environmental enrichment on cognitive function and hippocampal NGF in the non-handled rats. *Behav Brain Res, 103,* 63-70.

Polley, D., Kvasnák, E., Frostig, R. (2004). Naturalistic experience transforms sensory maps in the adult cortex of caged animals. *Nature, 429,* 67-71.

Prathanee, B., Purdy, S., Thinkhamrop, B., Chaimay, B., Ruangdaraganon, N., Mo-suwan, L., Phuphaibul, R. (2009). Early language delay and predictive factors in children aged 2 years. J *Med Assoc Thai, 92,* 930-8.

Ramamoorthy, R., Radhakrishnan, M., Borah, M. (2008). Antidepressant-like effects of serotonin type-3 antagonist, ondansetron: an investigation in behaviour-based rodent models. *Behav Pharmacol, 19,* 29-40.

Renner, M. (1987). Experiencee-dependent changes in exploratory behavior in the adult rat

(Rattus norvegicus): Overall activity level and interactions with objects. *J Compar Psych, 101,* 94-100.

Renner, M., Rosenzweig, M. Object interactions in juvenile rats (Rattus norvegicus): Effects of different experiential histories. *J Compar Psych, 100,* 229-36.

Rodgers, R., Lee, C., Sheperd, JK. (1992). Effects of diazepham on behavioural and antinociceptive responses to the elevated plus-maze in male mice depend upon treatment regimen and prior maze experience. *Psychopharmacology, 106,* 102-10.

Roy, V., Belzung, C., Delarue, C., Chapillon, P. (2001). Environmental enrichment in BALB/c mice: Effects in classical test of anxiety and exposure to a predatory odor. *Physiol Behav, 74,* 313-320.

Schrijver, N., Bahr, N., Weiss, I., Würbel, H. (2002). Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. *Pharmacol Biochem Behav, 73,* 209-24.

Schulz, D., Buddenberg, T., Huston, J. (2007). Extinction-induced "despair" in the water maze, exploratory behavior and fear: effects of chronic antidepressant treatment. *Neurobiol Learn Mem, 87,* 624-34.

Schulz, D., Huston, J., Buddenberg, T., Topic, B. (2007). "Despair" induced by extinction trials in the water maze: relationship with measures of anxiety in aged and adult rats. *Neurobiol Learn Mem, 87,* 309-23.

Schulz, D., Topic, B., De Souza, M., Huston, J. (2004). Extinction-induced immobility in the water maze and its neurochemical concomitants in aged and adult rats: a possible model for depression? *Neurobiol Learn Mem, 82,* 128-41.

Simon, P., Dupuis, R., Costentin, J. (1994). Thigmotaxis as an index of anxiety in mice: Influence of dopaminergic transmissions. *Behav Brain Res, 61,* 59-64.

Tabachnick, B., Fidell, L.. (2006). *Experimental design using ANOVA.* California: Thomson.

Topic, B., Huston, J., Namestkova, K., Zhu, S., Mohammed, A., Schulz, D. (2008). Extinction-induced ''despair" in aged and adult rats: Links to neurotrophins in frontal cortex and hippocampus. Neurobiol Learn Mem, 90, 519-526.

Treit, D., Fundytus, M. (1988) Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacol Biochem Behav, 31,* 959-62.

Tsai, P., Oppermann, D., Stelzer, H., Mähler, M., Hackbarth, H. (2003). The effects of different rack systems on the breeding performance of DBA/2 mice. *Lab Anim, 37,* 44-53.

Van der Borght, K., Wallinga, A., Luiten, P., Eggen, B., Van der Zee, E. (2005). Morris water maze learning in two rat strains increases the expression of the polysialylated form of the neural cell adhesion molecule in the dentate gyrus but has no effect on hippocampal neurogenesis. *Behav Neurosci, 19,* 926-32.

Van Praag, H., Kempermann, G., Gage, F. (2000). Neural consequences of environmental enrichment. *Nature Reviews Neuroscience, 1,* 191-8.

Van Waas, M., Soffie, M. (1996). Differential environmental modulations on locomotor activity, exploration and spatial behaviour in young and old rats. *Physiol Behav, 59,* 265-271.

Welberg, L., Thrivikraman, K., Plotsky, P. (2006). Combined pre- and postnatal environmental enrichment programs the HPA axis differentially in male and female rats. *Psychoneuroendocrinology, 31,* 553-64.

Whishaw, I., Sutherland, R., Kolb, B., Becker, J. (1986). Effects of neonatal forebrain noradrenaline depletion on recovery from brain damage: Performance on a spatial navigation task as a function of age of surgery and postsurgical housing. *Beh Neural Biol, 46,* 285-307.

Zhu, S., Yee, B., Nyffeler, M., Winblad, B., Feldon, J., Mohammed, A. (2006). Influence of

differential housing on emotional behaviour and neurotrophin levels in mice. *Behav Brain Res, 169,* 10-20.

Table 1

Results of gestational and postpartum EPM analyses

Measure	Figure	F value	P value
Gestational EPM test			
Main effects of interval			
closed arm frequency	fig 4E	F (2,12) = 4.675	p = 0.032*
open arm frequency	fig 4F	F (2, 12) = 11.875	p = 0.001*
rearing frequency	fig 5D	F (2, 12) = 4.783	p = 0.030*
grooming frequency	fig 5E	F (1.5, 9.2) = 9.605	p = 0.008*
edge duration	fig 5C	F (2, 12) = 4.485	p = 0.035*
edge frequency	fig 5F	F (2, 12) = 19.643	p < 0.001
Main effects of housing group			
grooming frequency	fig 5E	F (1, 6) = 16.164	p = 0.007*
Postpartum EPM test			
Main effects of interval			
closed arm duration per entry	fig 6A	F (1.6, 12.6) = 4.431	p = 0.042*
open arm duration per entry	fig 6B	F (2, 16) = 5.145	p = 0.019*
closed arm frequency	fig 6E	F (2, 16) = 35.588	p < 0.001*
open arm frequency	fig 6F	F (2, 16) = 11.021	p = 0.001*
rearing duration	fig 7A	F (2, 16) = 8.996	p = 0.002*
rearing frequency	fig 7D	F (2, 16) = 5.297	p = 0.017*
edge duration	fig 7C	F (2, 16) = 6.377	p = 0.009*
edge frequency	fig 7F	F (2, 16) = 13.474	p < 0.001*
Main effects of housing group			
rearing frequency	fig 7D	F (1, 8) = 5.597	p = 0.046*
grooming duration	fig 7B	F (1, 8) = 7.717	p = 0.024*
Interaction effect: interval x group			
open arm duration per entry	fig 6B	F (2, 16) = 4.043	p = 0.038*

Table 2

Results of EPM gestational and postpartum comparisons

Measure	F value	P value
Main effects of interval		
closed arm frequency	F (2, 12) = 14.048	p = 0.001*
open arm frequency	F (2, 12) = 20.626	p < 0.001*
rearing frequency	F (2, 12) = 6.531	p = 0.012*
grooming frequency	F (2, 12) = 4.563	p = 0.034*
edge duration	F (2, 12) = 6.319	p = 0.013*
edge frequency	F (2, 12) = 44.281	p < 0.001*
Main effects of housing group		
closed arm frequency	F (1, 6) = 15.240	p = 0.008*
grooming frequency	F (1, 6) = 6.600	p = 0.042*
Main effect of period		
edge duration	F (1, 6) = 18.040	p = 0.005*
Interaction effect: interval x period		
rearing duration	F (2, 12) = 3.962	p = 0.048*

Figure Captions

Fig. 1. The top part of the figure shows a photograph of the social colony apparatus. It consisted of six standard-size rat cages surrounding a larger custom made four-level middle cage; all standard cages were connected to adjacent cages and the larger middle cage by tubes to allow movement around the colony. See text for additional details. The bottom part of the figure shows the time line of events. The gestational period was 23 days. Gestational EPM test was conducted on GD 19. Weaning occurred gradually, beginning on APND 20 and ending on APND 26. Postpartum EPM procedure was conducted on APND 27 and the MWM test on APND 38.

Fig. 2. The plot tracks the daily mean gestational and postpartum body weight (±SEM) based on housing condition. Social colony data are shown as filled circles and control data as unfilled circles.

Fig. 3. Litter characteristic comparisons between the social colony and control groups. Plot A shows the mean litter weight (± SEM), plot B, the mean number of pups per litter (± SEM), and plot C, the average pup weight per litter (± SEM). Social colony data appear as dark grey bars and the control data as light grey bars. The symbol * indicates a statistically significant housing group difference at $p < 0.05$.

Fig. 4. The figure shows the gestational EPM open (right side) and closed (left side) arm measures. Graphs A and B plot average duration per entry, graphs C and D, mean duration, and graphs E and F, mean frequency. Error bars depict the SEM. Social colony data are represented by filled circles and control data by unfilled circles. Values that appear in the top right side of each plot refer to the probability obtained in the analyses of group differences in slope. The alpha level was set at .004 to correct for multiplicity.

Fig. 5. The figure shows the gestational EPM measures of rearing (left side), grooming (middle), and edge (right side) behaviours. The top plots are associated with mean duration scores and the

bottom plots, mean frequency scores. Error bars refer to the SEM. Social colony data are represented by filled circles and control data by unfilled circles. Values that appear in the top right side of each plot refer to the probability obtained in the analyses of group differences in slope. The alpha level was set at .004 to correct for multiplicity.

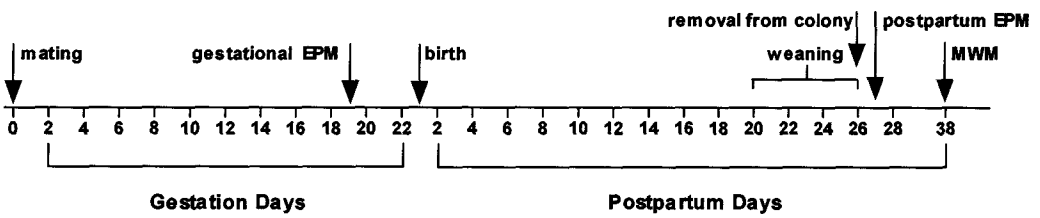
Fig. 6. The figure shows the postpartum EPM open (right side) and closed (left side) arm measures. Graphs A and B plot average duration per entry, graphs C and D, mean duration, and graphs E and F, mean frequency. Error bars depict the SEM. Social colony data are represented by filled circles and control data by unfilled circles. Values that appear in the top right side of each plot refer to the probability obtained in the analyses of group differences in slope. The alpha level was set at .004 to correct for multiplicity.

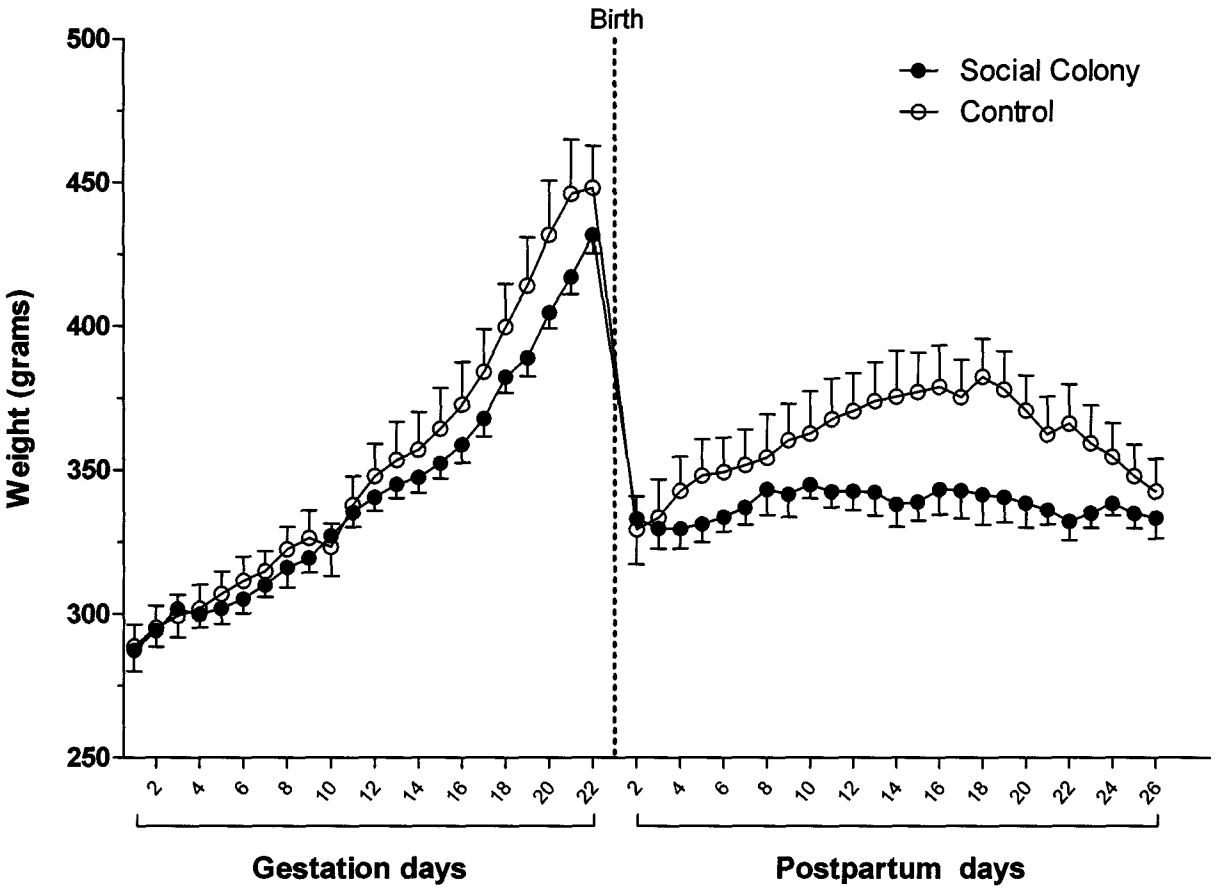
Fig. 7. The figure shows the postpartum EPM measures of rearing (left side), grooming (middle), and edge (right side) behaviours. The top plots are associated with mean duration scores and the bottom plots, mean frequency scores. Error bars refer to the SEM. Social colony data are represented by filled circles and control data by unfilled circles. Values that appear in the top right side of each plot refer to the probability obtained in the analyses of group differences in slope. The alpha level was set at .004 to correct for multiplicity.

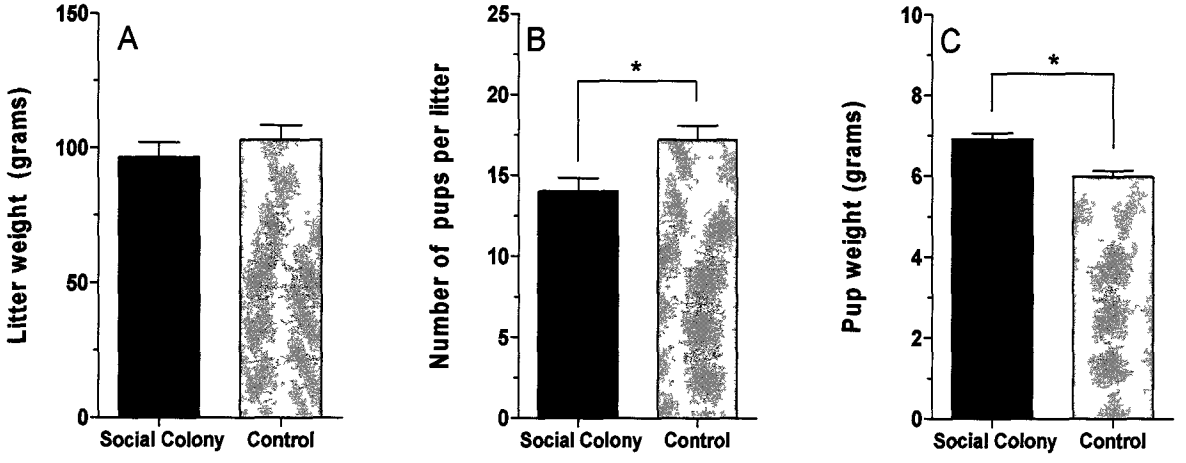
Fig. 9. Mean latency to locate the platform in the MWM test over 16 trials, divided into four blocks. Social colony data are represented by filled circles and control data by unfilled circles. Error bars refer to the SE.

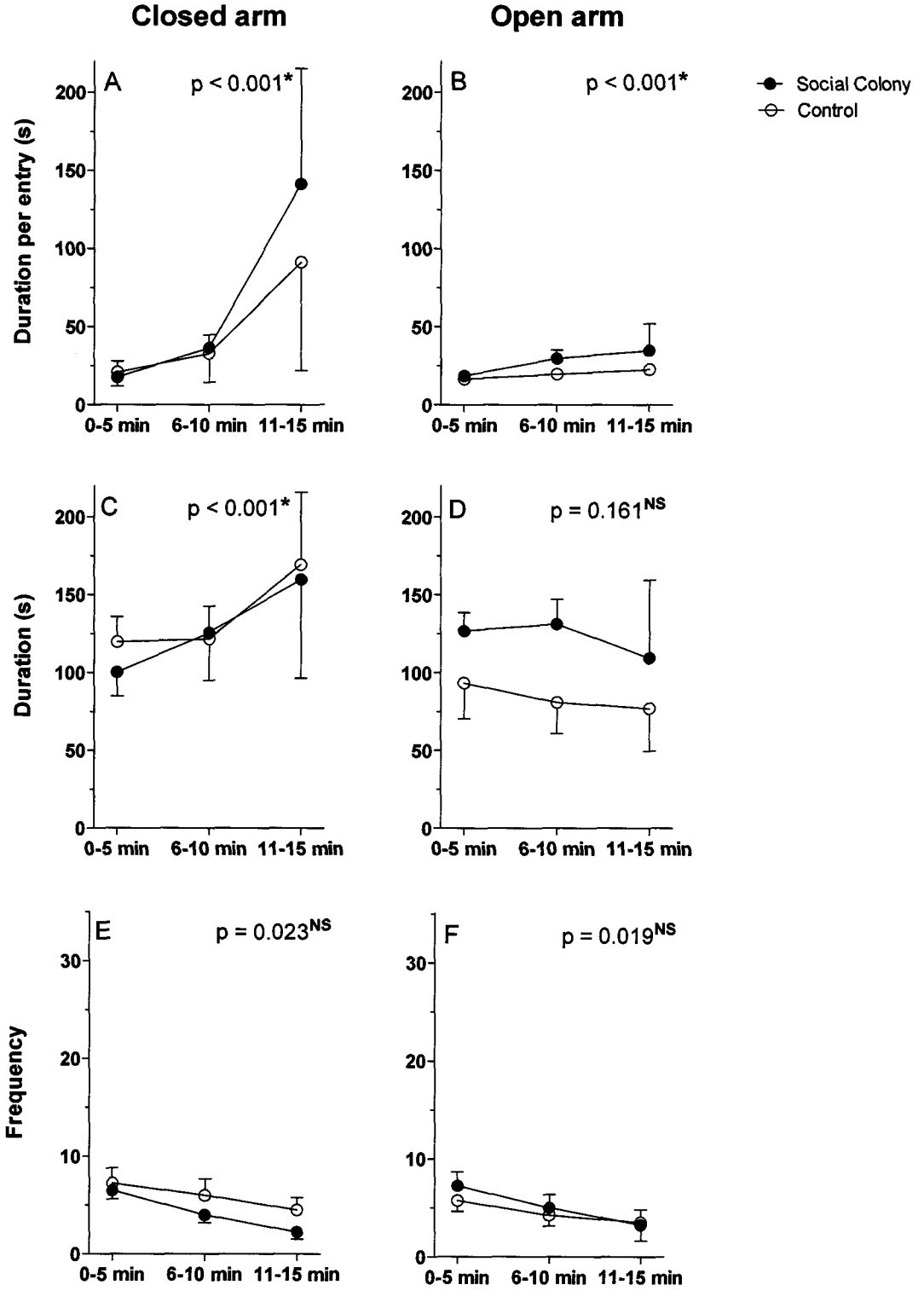
Fig. 10. The figure shows group data associated with several MWM measures. On the left side, location of platform quadrant appears expressed as mean duration per entry (plot A), duration (plot B), and frequency (plot C). On the right side, the frequency to approach the platform position (plot B), frequency to approach the middle of the maze (plot D), and the duration of thigmotactic swimming (plot F) are shown. Social colony data are represented by dark grey bars

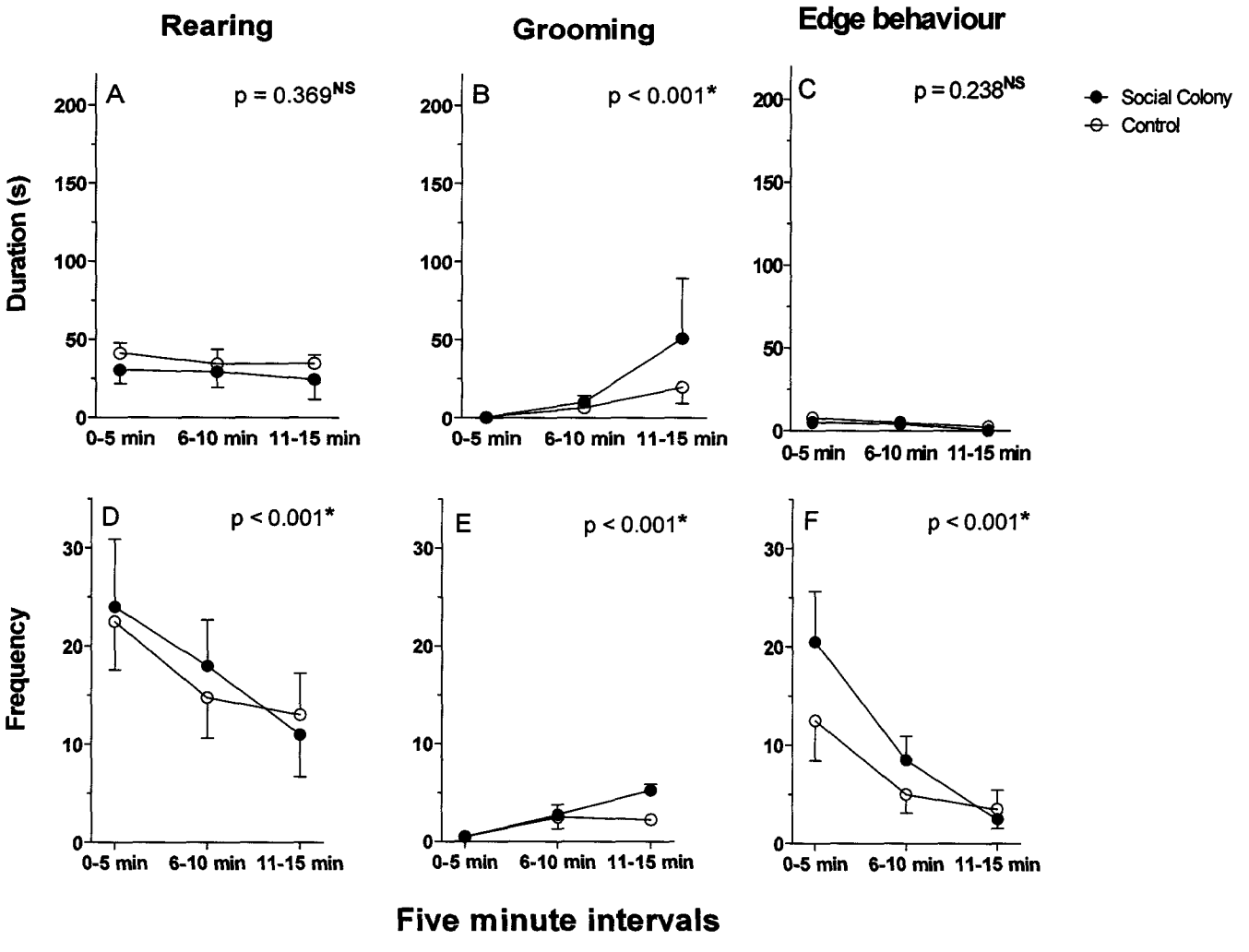
and control data by light grey bars. Error bars depict the SEM. Significant housing group differences are indicated by an *; $p \leq 0.05$.

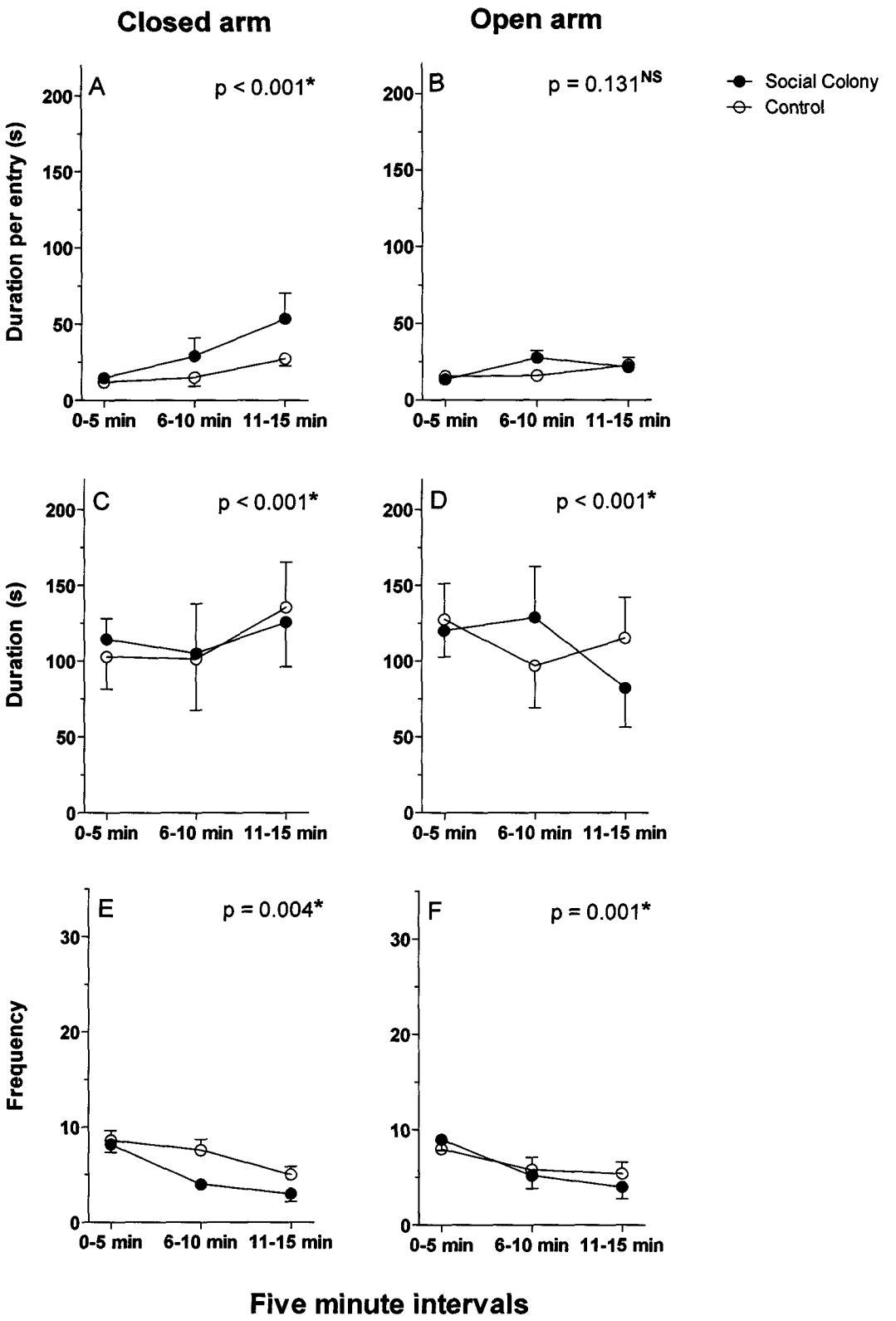

mating
gestational EPM
birth
removal from colony
weaning
postpartum EPM
MWM
0 2 4 6 8 10 12 14 16 18 20 22 2 4 6 8 10 12 14 16 18 20 22 24 26 28 38
Gestation Days
Postpartum Days

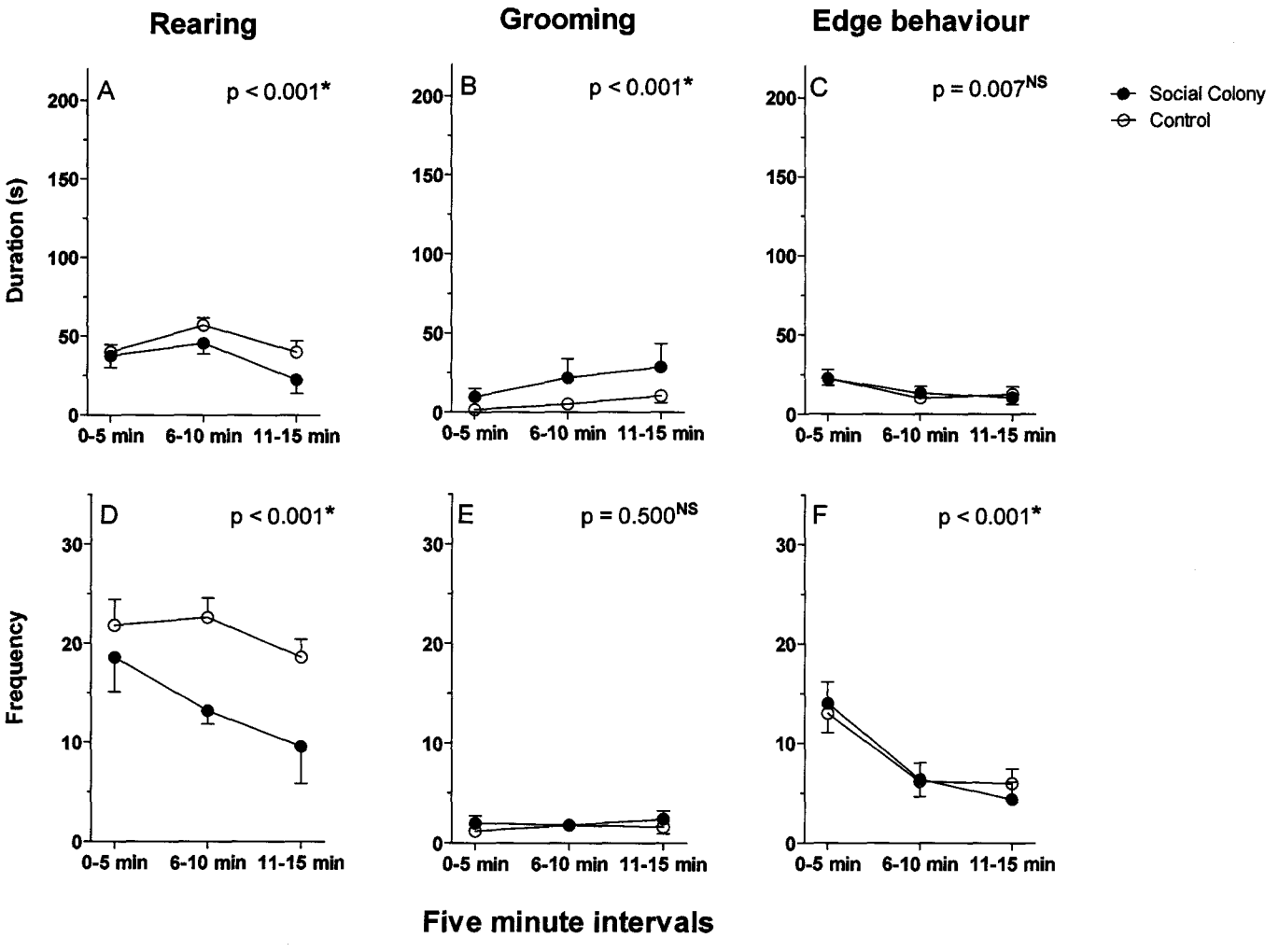

Birth
Social Colony
Control
Weight (grams)
500
450
400
350
300
250
2 4 6 8 10 12 14 16 18 20 22
2 4 6 8 10 12 14 16 18 20 22 24 26
Gestation days
Postpartum days

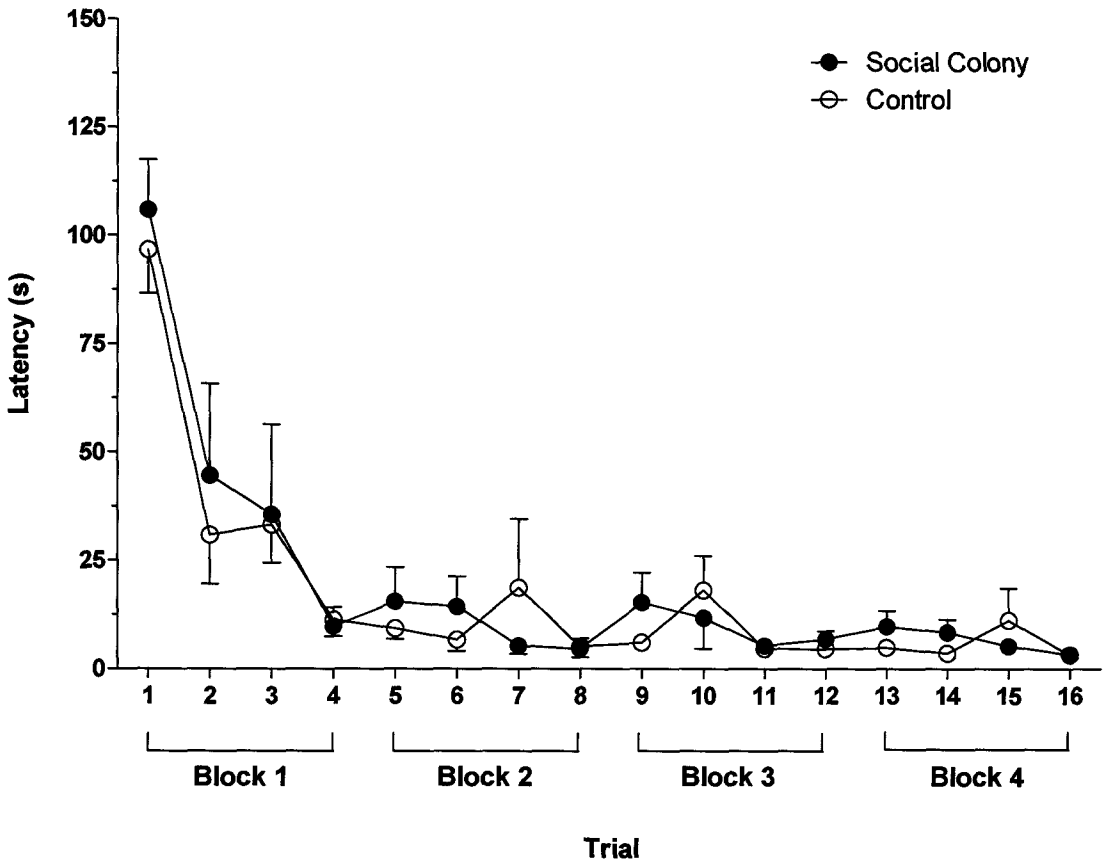

A
Litter weight (grams)
150
100
50
0
Social Colony
Control
B
Number of pups per litter
25
20
15
10
5
0
*
Social Colony
Control
C
Pup weight (grams)
10
8
6
4
2
0
*
Social Colony
Control

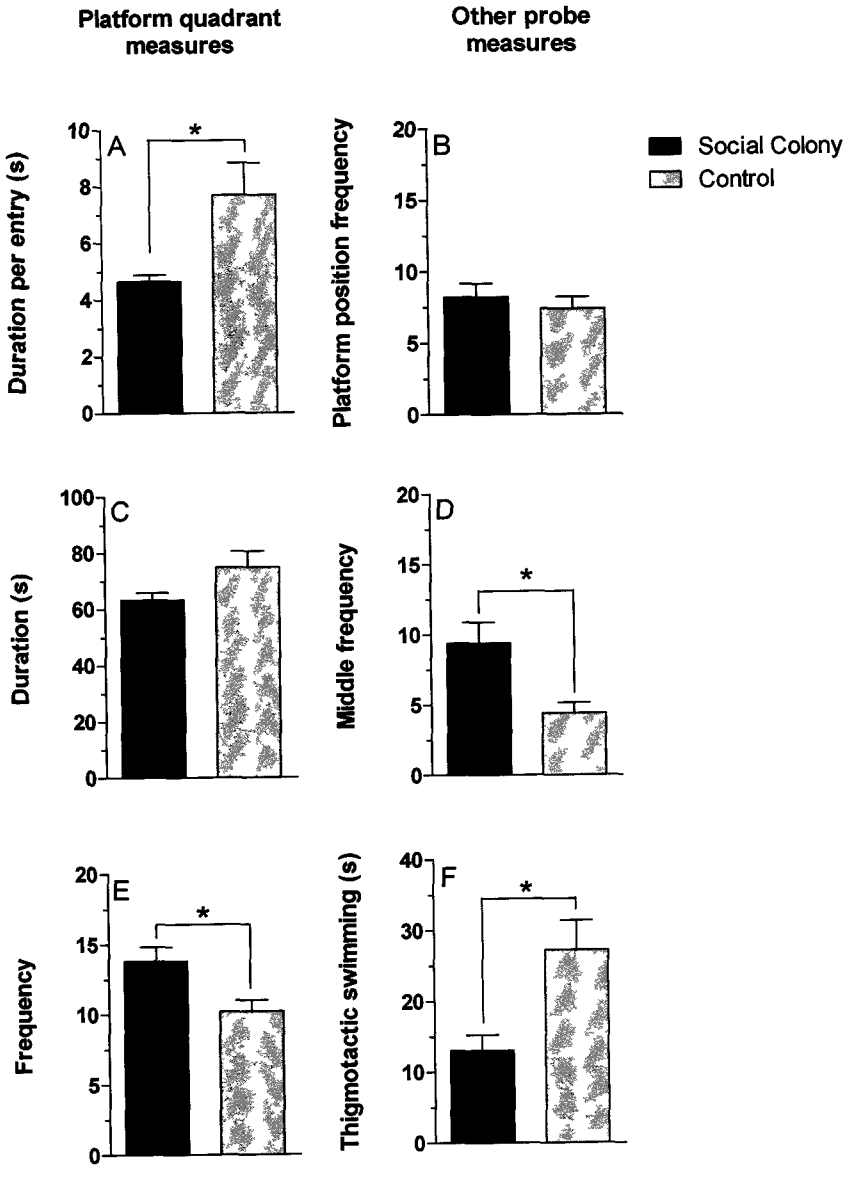

Closed arm
Open arm
A
p < 0.001*
B
p < 0.001*
Social Colony
Control
Duration per entry (s)
C
p < 0.001*
D
p = 0.161NS
Duration (s)
E
p = 0.023NS
F
p = 0.019NS
Frequency
0-5 min
6-10 min
11-15 min
Five minute intervals


Rearing
Grooming
Edge behaviour
A
p = 0.369NS
B
p < 0.001*
C
p = 0.238NS
Social Colony
Control
Duration (s)
200
150
100
50
0
0-5 min
6-10 min
11-15 min
D
p < 0.001*
E
p < 0.001*
F
p < 0.001*
Frequency
30
20
10
Five minute intervals


Closed arm
Open arm
A
p < 0.001*
B
p = 0.131NS
Social Colony
Control
Duration per entry (s)
200
150
100
50
0
0-5 min
6-10 min
11-15 min
C
p < 0.001*
D
p < 0.001*
Duration (s)
E
p = 0.004*
F
p = 0.001*
Frequency
30
20
10
Five minute intervals


Rearing
Grooming
Edge behaviour
A
p < 0.001*
B
p < 0.001*
C
p = 0.007NS
D
p < 0.001*
E
p = 0.500NS
F
p < 0.001*
Duration (s)
Frequency
0-5 min
6-10 min
11-15 min
Social Colony
Control
Five minute intervals


Social Colony
Control
Latency (s)
150
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Block 1
Block 2
Block 3
Block 4
Trial


Platform quadrant measures
Other probe measures
Social Colony
Control
A
B
C
D
E
F
Duration per entry (s)
Platform position frequency
Duration (s)
Middle frequency
Frequency
Thigmotactic swimming (s)
*