MATERNAL CARE AFFECTS MALE AND FEMALE OFFSPRING
WORKING MEMORY AND STRESS REACTIVITY

by

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ABSTRACT

Variations in maternal care affect the development of individual differences in learning and memory and neuroendocrine responses to stress in adult male offspring, but it is not known how variations in maternal care affect adult female offspring. The present study investigated the performance of adult Sprague-Dawley male and female offspring exposed to either low or high levels of maternal licking/grooming on a spatial learning and memory task (Experiment 1) and the effects of acute stress on corticosterone levels and spatial memory performance (Experiment 2). In Experiment 1 rats were trained for 24 days on the spatial working/reference memory version of the radial arm maze (RAM). In experiment 2, rats were trained on the same RAM task, exposed to an acute stress, and the effect of stress on corticosterone levels and subsequent spatial memory was examined. In experiment 1, adult female offspring of low licking/grooming dams had enhanced working memory compared to all other groups. In experiment 2, all groups of male and female offspring had enhanced working memory 24h after exposure to acute 2h restraint stress while reference memory was enhanced after stress in male and female offspring of low licking/grooming dams. Furthermore, female offspring of low licking/grooming dams showed the largest corticosterone response to the acute restraint stress compared to all other groups. Male offspring of low licking/grooming dams showed a flattened corticosterone response to stress. Thus variations in maternal care differentially affect working memory and stress reactivity in male and female offspring.

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CO-AUTHORSHIP STATEMENT

Jodi Pawluski assisted with blood collection and proofread the manuscript.

1. INTRODUCTION¹

Hippocampus-dependent learning and memory performance is affected by the sex of the subject [1], early life experience [2], and acute stress [1]. Generally, males outperform females in spatial learning and memory tasks, and acute stress impairs spatial learning and memory in males but not females [1]. Sex differences in spatial memory performance favoring males depend on a multitude of factors including hormonal state [3], pre-training [4], and strategies used to solve the task [5]. In addition, sexually-dimorphic performance on the Morris water maze and the radial arm maze is influenced by species and strain differences [6,7], age [8], diet [9], procedural differences [6,10], cues [5], and rearing conditions [10-12]. Furthermore, males born to high licking/grooming dams outperform males born to low licking/grooming dams in spatial learning and memory tasks in adulthood, although to date no work has looked at the effects of maternal licking/grooming on spatial learning and memory performance of adult female offspring.

Exposure to acute stress results in sex differences in cognitive performance, although the direction of this sex difference depends on the specific stressor and the cognitive task. Conrad et al [1] found that exposure to restraint stress impaired the spatial memory of male rats in the Y-maze, in which performance depends on the hippocampus [13], but enhanced the spatial memory of female rats on the same task. On the other hand, studies from Tracey Shors' laboratory have found that exposure to electric shock enhanced trace eye-blink conditioning (which is dependent on the integrity of both the

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hippocampus and cerebellum [14,15]) in adult male rats, and impaired trace eye-blink conditioning in adult female rats [16-18].

There is also a well-known sex difference in the neuroendocrine response to acute stress with female rats responding more rapidly and with higher corticosterone levels than male rats [19,20]. Furthermore, adult male offspring of high licking/grooming dams have been shown to have decreased corticosterone levels, decreased adrenocorticotrophic hormone levels, and increased hippocampal glucocorticoid receptor mRNA expression in response to stress (for review see [21]) compared to male offspring of low licking/grooming dams [22,23,24]. However, to date there is no literature concerning the effects of maternal care on the neuroendocrine response of adult female offspring to acute stress.

Naturally occurring variations in maternal care during the early postnatal period have also been shown to alter cognitive development in adult male offspring, whereas less is known about how maternal care affects adult female offspring [2,22]. In the rat, adult male offspring receiving high levels of licking/grooming and arched-back nursing showed enhanced learning and memory in both the Morris water maze and object recognition task than offspring receiving low levels of licking/grooming and arched-back nursing [2,25]. These effects were not due to genetic factors, but instead due to the quantity of licking/grooming received [23]. In all of these studies on maternal care, only adult male Long Evans hooded offspring were used, therefore it is not clear whether these findings extend to adult female offspring and/or other strains of rat.

In addition to the effects of maternal care on hippocampal spatial learning and memory and neuroendocrine response to acute stress, male offspring of high

licking/grooming dams have decreased startle responses, increased open-field exploration, and exhibit shorter latencies to eat food after exposure to acute stress [26,27]. Furthermore, adult male offspring who received low levels of maternal licking/grooming were found to be more fearful in a novel environment than were adult male offspring who received high levels of maternal licking/grooming [26]. Although no studies have looked at sex differences in the effects of maternal behavior on resilience to stress in the offspring, as reported earlier there is evidence in the literature that a sex difference exists in both spatial learning and memory and the cognitive response to acute stress [1,16,17,28]. This suggests a possible sex difference in the effects of maternal care and the development of sexually dimorphic alterations in spatial learning and memory following acute stress.

Therefore, the present study investigated the performance of adult male and female offspring exposed to low and high levels of maternal licking/grooming on a spatial learning and memory task and the effects of acute stress on spatial performance. Experiment 1 investigated hippocampus-dependent spatial learning and memory performance in adult male and female offspring exposed to low and high levels of maternal licking/grooming. Experiment 2 investigated the role of acute restraint stress on corticosterone response and hippocampus-dependent spatial learning and memory performance in adult male and female offspring exposed to low and high levels of maternal licking/grooming.

The spatial working/reference version of the radial arm maze was used in both Experiments 1 and 2 as it allows for the simultaneous assessment of working memory and reference memory [29]. Working memory is defined as the manipulation and

retrieval of trial-unique information that is used to guide prospective action and reference memory is defined as long-term stable memory [29]. In Experiment 2, the neuroendocrine response to acute restraint stress was assessed by measuring serum corticosterone (CORT) levels at various times after the onset of restraint stress. In Experiment 1, it was expected that male offspring of high licking/grooming dams would have enhanced working and reference memory performance compared to all other offspring. In Experiment 2, it was expected that working and reference memory performance would be enhanced following exposure to acute restraint stress and the effects may be dependent on the sex of the subject. However, to date it is not known how stress may regulate spatial memory performance in male and female offspring of high or low licking/grooming dams. It was expected that offspring of high licking/grooming dams would have a decreased corticosterone response to the restraint stress, and that a sex difference would exist in the cognitive and neuroendocrine responses to the acute restraint stress.

2. METHODS

2.1. Animals

Thirty male and thirty female offspring of 6 Sprague-Dawley dams (UBC Animal Care Facility, Vancouver, Canada) were used. Pups were weaned on postnatal day 21 and housed in same-sex, same litter groups until postnatal day 35 when rats were singly housed in opaque polyurethane bins (48 x 27 x 20 cm) with aspen chip bedding. Rats were given tap water and Purina rat chow ad libitum (except where otherwise noted) and maintained in a 12h:12h light/dark cycle (lights on 7:00 am). Rats were left undisturbed, except for weekly cage changing, until one week before testing (postnatal day 70) at which time rats were handled for 10 minutes each day. All protocols were in accordance with ethical guidelines set by the Canada Council for Animal Care and were approved by the University of British Columbia Animal Care Committee.

2.2. Breeding

For breeding, one dam and one stud male were paired in a wire mesh cage. Upon release of a vaginal plug dams were individually housed in clear polyurethane bins until birth. One day after birth (birth day = day 0), each litter was culled to 5 male and 5 female offspring. The dam and pups were housed in clear polyurethane bins until day 8 postpartum at which time they were housed in large opaque polyurethane bins (51 x 41 x 22cm) until weaning (postpartum day 21).

2.3. Maternal Behavior testing

For observation of maternal behavior, dam and pups were left undisturbed in the cage. Observations were made every 5 seconds for 10 minutes three times a day (between 8-9:30a.m., 11-12:30p.m. and 2-4p.m) on days 2 to 8 post-parturition. The duration as

well as the frequency of each specific maternal behavior was recorded. Maternal behavior was not assessed on day 1 post-parturition because pups were culled on this day. The following maternal behaviors were assessed as previously described [30]:

licking/grooming (body licking and genital licking with the dam off the pups);
licking/grooming and nursing; arched-back nursing; “blanket” nursing; passive nursing;
and time off pups. Data for each behavior was compiled across all 21 test periods as the number of 5 second samples spent in a scored behavior. For the duration of specific maternal behavior, frequencies were multiplied by 5 seconds in order to ascertain the amount of time engaged in a scored behavior. In addition, all licking behaviors (licking/grooming, licking/grooming and nursing) were summed to create a single licking/grooming (LG) variable as in previous studies [22].

Dams, and their offspring, were designated as High-LG if their total licking/grooming behavior was half a standard deviation above the cohort mean. Dams were designated as Low-LG if their total licking/grooming behavior was half a standard deviation below the cohort mean. The dam cohort for this study consisted of 8 females. No more than three male and three female offspring per group were drawn from any single litter for a total of 9 High-LG males, 9 High-LG females, 9 Low-LG males, and 9 Low-LG females for experiment 1. The remaining offspring from each litter were used for experiment 2 for a total of 6 High-LG males, 6 High-LG females, 6 Low-LG males, and 6 Low-LG females.

2.4. Spatial Learning and Memory

Radial arm maze testing began when adult offspring were approximately 80 days old. Spatial learning and memory was investigated using the spatial working/reference

memory version of the radial arm maze [29,30]. The eight-arm radial arm maze was elevated 80 cm from the floor, with arms (53 cm long x 10 cm wide) projecting at equal angles from an octagonal center platform (36 cm in diameter). It was located in a room with multiple extra-maze cues that remained in constant positions throughout the duration of the experiment. The maze was randomly rotated twice a week to minimize the use of intra-maze cues. In order to facilitate comparison with previous literature, rats were tested during the light phase of the light:dark cycle. Testing during this phase was also done in order to minimize the effects of endogenous levels of corticosterone which follow a diurnal rhythm and are at relatively low levels during the light phase but increase substantially during the dark phase [31]. In experiment 1, female rats were tested between 2 pm and 4 pm and male rats were tested between 12 pm and 2 pm. In experiment 2, female rats were tested between 8 am and 10 am and male rats were tested between 10 am and 12 pm. However, the two hour time window for testing each group was substantially reduced with training. This difference in timing was necessary in order to separately test male and female groups to limit the potential confound of opposite sex odors to influence performance. All rats were introduced to the food reward (Kellogg's Fruit Loops Cereal, Battle Creek MI) in their home cages for 3 days prior to habituation. As well, rats were food deprived to 85% of their body weight beginning on the first day they received the food reward. A calculated amount of food pellets was placed into the cage of the rat after radial arm maze testing each day based on current weight of the rat and percentage of weight loss from previous day. Food deprivation was conducted in such a way that weight loss occurred gradually. As well natural growth with weight gain

was taken into account when determining the amount of food to be given to each individual rat and rats were allowed growth of 3 g/week.

All rats were habituated to the maze for a total of 20 minutes over three days. Rats were placed on the center platform and allowed to explore freely for the allotted amount of time. The maze was thoroughly cleaned with 70% ethyl alcohol after each rat. After habituation, each rat was randomly assigned a separate pattern of baited and non-baited arms (four baited arms out of eight possible arms) that remained constant for the duration of the experiment. Rats were then shaped for 3 days which consisted of the rat being placed on the center of the platform with access to all arms. The assigned arms were each baited with a Fruit Loop divided into 4 equal parts placed at equidistant intervals along the arm. Each shaping session took place until either 10 minutes had elapsed or the rat had entered all the baited arms. An arm was considered entered when the front legs of the rat crossed halfway down the distance of the arm. Training sessions, one per day, consisted of the rat being released on the center of the platform and remaining on the maze until all baited arms had been entered or until 10 minutes had elapsed. The end of the baited arm contained 1/4 of a Fruit Loop.

In experiment 1, rats were trained for 24 consecutive days to assess the role of maternal variation in licking/grooming during development on sex differences in spatial learning and memory performance. In experiment 2, different rats from those in Experiment 1 were trained for 7 consecutive days, exposed to an acute restraint stress on the 8th day, and then trained for 1 more day on the radial arm maze. This was done to assess the role of stress on spatial learning and memory performance in adult offspring of low and high licking/grooming dams.

During each training session, rats could make three types of errors: 1) reference memory errors (RME) defined as entries into non-baited arms, 2) working memory errors (WME) defined as repeat entries into baited arms, and 3) working/reference memory errors (W/RME) defined as repeat entries into non-baited arms. For each rat, total RME, WME, W/RME, number of days to reach criterion (defined as no more than 2 errors per day for 2 consecutive days), total number of errors to criterion, and average latency to reach each arm (seconds) were also calculated.

2.5. Estrous Cycle

Rats in proestrus are more likely to make spatial errors in a Morris water maze [32]. For both experiments 1 and 2, proestrus was determined for female rats from daily vaginal smears done following each session on the radial arm maze, beginning the first day of habituation. Smears were taken by placing a cotton swab in the vagina and smearing the contents of the swab on a plain slide. Slides were examined under 10x objective and proestrus was determined when a majority of cells evident in the vaginal mucus (approximately 70%) were nucleated epithelial cells.

2.6. Restraint Stress (Experiment 2)

In experiment 2, rats were exposed to an acute restraint stress on day 8 of maze training, which consisted of offspring being restrained in individual polyethylene restraining tubes (6 cm diameter x 15 cm long for females; 6 cm diameter x 20 cm long for males, with four holes in the front and an opening at the back for the tail) for 120 minutes. During restraint, rats were kept in a novel room that was brightly lit. Rats were released after 120 min restraint and placed back into their home cages and kept in the

novel room for three hours [28] before being returned to colony rooms. Rats were not trained on the RAM on day 8.

2.7. Corticosterone measures and assay (Experiment 2)

In experiment 2 serial blood samples were collected from tail nicks. Blood was sampled at baseline and then at 30, 60, and 120 minutes after the onset of restraint. Baseline corticosterone levels for all rats were determined by sampling tail blood within 3 minutes of entering the colony room. Baseline blood samples of offspring were taken in one morning between the hours of 8am and 11:30am. Rats were placed into restraint tubes immediately after baseline blood sample was collected.

Blood samples were stored overnight at 4°C and centrifuged at 10×g for 15 min. Total serum corticosterone levels were assayed by radioimmunoassay using the Double Antibody ¹²⁵I RIA Kit for rat corticosterone available commercially from MP Biomedicals (Orangeburg, NY) with an average intra-assay coefficient of variation of 3.5% and a sensitivity of 7.7 ng/ml. For the assays, the standard curve ED20 was between 818.4 and 850.1ng/ml, ED50 was between 150.3 and 151.9 ng/ml, and for ED80 was between 27.96 and 29.6 ng/ml.

2.8. Data Analyses

2.8.1. Maternal Behavior

To determine differences in duration of maternal care, t-tests were performed between duration of a maternal behavior (licking/grooming behaviors, arched-back nursing, “blanket” nursing, passive nursing, and time off pups) and group (High-LG and Low-LG dams).

2.8.2. Experiment 1

For Experiment 1, separate repeated-measures analysis of variance (ANOVA)s were calculated for the number of RME, WME, and W/RME and latency to reach an arm with group (High-LG, Low-LG) and sex (male, female) as between-subjects factors and days (24 test days) as within-subjects factors. Two-way ANOVAs were conducted on number of days to reach criterion and total number of errors to reach criterion, with group (High-LG, Low-LG) and sex (male, female) as between-subjects factors. Potential litter effects were also examined using litter as a nested factor within group in all the above comparisons. To determine the potential effects of proestrus, an analysis of covariance was calculated on number of errors across days using proestrus as the covariate. An independent-samples t-test was performed to see whether differences in the total number of days spent in proestrus during testing existed between female offspring of high and low licking/grooming dams. To determine whether there was a relationship between duration of maternal behavior (LG) and learning and memory performance in the RAM, Pearson product-moment correlations were conducted between duration of licking/grooming and total RME or total WME errors in offspring of high licking/grooming dams and in offspring of low licking/grooming dams.

2.8.3. Experiment 2

For Experiment 2, in order to confirm there were no significant differences between groups in radial arm maze performance prior to the acute restraint stress, separate repeated-measures ANOVAs were calculated on number of RME, WME, and W/RME and latency to reach an arm with group (High-LG, Low-LG) and sex (male, female) as between-subjects factors and days (1 to 6) as within-subjects factors.

Furthermore, separate repeated-measures ANOVA were conducted on WME, RME, W/RME, and latency to reach an arm with group (High-LG, Low-LG) and sex (male, female) as between-subjects factors and days (pre-stress, post-stress) as within-subjects factor. Potential litter effects were also examined using litter as a nested factor within group in all the above comparisons. To determine the potential effects of proestrus, an analysis of covariance was calculated on number of errors across days using proestrus as the covariate. An independent-samples t-test was performed to see whether differences in the total number of days spent in proestrus during testing existed between female offspring of high and low licking/grooming dams. Furthermore, serum corticosterone levels during restraint stress were analyzed using a repeated-measures ANOVA with group (High-LG, Low-LG) and sex as between-subjects factors and time (baseline, 30 min, 60 min, 120 min) as within-subject factor. Potential litter effects were also examined using litter as a nested factor within group in all the above comparisons. To determine whether there was a relationship between duration of maternal behavior (LG) and initial corticosterone response to the restraint stress, Pearson product-moment correlations were conducted between duration of licking/grooming and the difference between 30 minute corticosterone levels and baseline corticosterone levels.

In experiment 1 three rats (1 male from the High-LG group and two females from the Low-LG group) were excluded from all data analysis because for the majority of the training sessions (greater than 67%) they did not complete the maze within the 10 minute time limit. All post-hoc comparisons utilized Newman-Keuls post-hoc procedure unless otherwise specified.

3. RESULTS

3.1. Maternal Behavior

The dams' mean licking/grooming behavior was 1210.63 seconds with a standard deviation of 350.75 seconds across 7 days. Therefore, three dams were defined as High-LG who had licking/grooming behavior that was half a standard deviation above the mean (≥ 1385.98 sec) and three dams were defined as Low-LG had licking/grooming behavior that was half a standard deviation below the mean (≤ 1035.28 sec). As expected, High-LG dams had a significantly greater duration of licking/grooming behavior (mean $\pm$ SEM: $1511.67 \text{ sec} \pm 105.53 \text{ sec}$) than Low-LG dams ($825.00 \text{ sec} \pm 85.78 \text{ sec}$) ($t(4) = 5.05, p < .01$). There were no significant differences between High-LG dams and Low-LG dams on any other individual maternal behaviors measured ($.43 \leq p \leq .93$; see Table 1).

Table 1. The mean $\pm$ SEM duration in seconds high and low LG dams spent performing individual maternal behaviors. There were no significant differences between groups in any of the listed maternal behaviors.

Group	Arched-back nursing	"Blanket" nursing	Passive nursing	Time off pups
High-LG dams	1955.00 ± 810.56	6333.33 ± 1011.94	1846.67 ± 531.42	753.33 ± 226.74
Low-LG dams	1400.00 ± 287.79	7441.67 ± 754.32	2151.67 ± 386.84	776.67 ± 456.70

3.2. Experiment 1: Spatial learning and memory in adult offspring of high and low licking/grooming dams

3.2.1. Female offspring of low licking/grooming dams had significantly better working memory performance than female offspring of high licking/grooming dams.

Figure 1 displays the mean ($\pm$ SEM) number of (a) total working memory errors (WME) across the twenty-four days of training, and (b) WME arranged in blocks of four

days for male and female offspring of low and high LG dams. A repeated-measures ANOVA was conducted on WME, RME, and W/RME with group and sex as between-subjects factors and day (days 1 to 24) as within-subjects factor. For WME a significant two-way interaction between group and sex ($p \leq .01$) was found. Post-hoc tests revealed that female offspring of low LG dams made significantly fewer WME than female offspring of high LG dams ($p \leq .01$), male offspring of low LG dams ($p \leq .05$) and male offspring of high LG dams ($p \leq .05$). Female offspring of high LG dams did not significantly differ in number of WME from either male group ($p > .51$). There was also a significant main effect of group ($p \leq .05$), with offspring of low LG dams making fewer WME than offspring of high LG dams, and a significant main effect of day ($p \leq .001$). For RME and WRME there were significant main effects of day (both p 's $\leq .001$), indicating that as the days progressed there were fewer errors committed regardless of group. There were no other significant main effects or significant interactions (data not shown). There was also no significant effect of litter for WME, RME, or WRME (all p 's $\geq .20$).

A

B

Figure 1. Mean ($\pm$ SEM) number of (a) total working memory errors (WME) across the twenty-four days of training, and (b) WME arranged in blocks of four days for male and female offspring of high and low LG dams ($n = 7-9$). Though all groups committed fewer WME as the number of days increased only female offspring of low LG dams made significantly fewer WME than female offspring of high LG dams and tended to make fewer WME than male offspring of high and low LG dams.

3.2.2. Female offspring require fewer days to reach criterion than male offspring.

ANOVAs revealed a significant main effect of sex when conducted on number of days to reach criterion ($F(1, 29) = 13.19, p \leq .001$; Table 2) and on total errors to criterion ($F(1, 29) = 6.46, p \leq .02$; Table 2), with females requiring fewer days and making fewer errors to reach criterion. There were no other significant main effects or significant interactions ($.51 \leq p \leq .93$). There was also no significant effect of litter for number of days to reach criterion and total errors to criterion (all p 's $\geq .10$).

Table 2. The mean $\pm$ SEM number of days to reach criterion and number of total choices to criterion in high and low LG male and female offspring. There was a significant main effect of sex on number of days to reach criterion and total errors to criterion, with females requiring fewer days and making fewer errors to criterion. There were no other significant main effects or significant interactions.

Group	Days to criterion	Total errors to criterion
High-LG Males	19.25 $\pm$ 2.04	97.63 $\pm$ 13.40
Low-LG Males	18.44 $\pm$ 2.86	90.78 $\pm$ 13.94
High-LG Females	10.67 $\pm$ 2.33*	63.56 $\pm$ 18.00*
Low-LG Females	9.43 $\pm$ 2.06*	50.86 $\pm$ 8.14*

Data are presented as mean $\pm$ SEM. * denotes significantly different from males.

A repeated-measures ANOVA on latency to reach an arm revealed a significant main effect of day ($F(23, 667) = 8.91, p \leq .0001$), indicating that as the days progressed latency to reach an arm decreased. There were no other significant main effects or significant interactions ($.11 \leq p \leq .94$). There was also no significant effect of litter for latency to reach an arm ($p \geq .90$).

3.2.3. Proestrus did not account for differences in learning and memory performance.

Analysis of covariance on total number of errors with the number days spent in proestrus as a covariate across days did not significantly alter the above findings, as female offspring of low LG dams made significantly fewer WME than female offspring of high LG dams ($p \leq .02$). There was also no significant effect of the covariate ($p \leq .91$). In

addition, there was no significant difference in total number of days across testing spent in proestrus between female offspring of high and low LG dams ($p \leq .16$).

3.2.4. Increased licking/grooming is associated with decreased reference memory errors in offspring of high licking/grooming dams.

A significant negative correlation was found between duration of licking/grooming and total RME in the offspring of high LG dams ($r = -.49$, $p \leq .05$; Figure 2) but not in the offspring of low LG dams ($r = -.22$, $p \leq .39$). There were no other significant correlations ($p \geq .1$).

Figure 2. The association between duration of licking and grooming behavior of dams and total reference memory errors (RME). There was a significant negative correlation between duration of LG and total RME in offspring of high LG dams ($r = -.49$), indicating that more licking and grooming received by male and female offspring is coincident with decreased RME errors. There was no significant

correlation between duration of LG and total RME in offspring of low LG dams ($r = -.22$).

3.3. Experiment 2: The effect of restraint stress on spatial learning and memory

In order to confirm there were no significant differences between groups in radial arm maze performance prior to the acute restraint stress, a repeated measures analysis of variance (ANOVA) was conducted on WME, RME, and WRME with group and sex as between subjects factors and day (day 1 to day 6) as within-subjects factor. For WME there was a significant interaction between day and group ($F(5, 100) = 2.70, p \leq .05$; Figure 3). Post-hoc tests revealed that offspring of low and high LG dams differed in WME only on day 3 ($p \leq .05$), with offspring of low LG dams committing more WME than offspring of high LG dams. There was also a significant main effect of day ($F(5, 100) = 3.59, p \leq .01$). There were no other significant main effects or significant interactions ($.15 \leq p \leq .75$). There were no significant main effects or significant interactions for RME ($.18 \leq p \leq .97$) or for WRME ($.09 \leq p \leq .69$). There was also no significant effect of litter for WME, RME, or W/RME (all p 's $\geq .20$).

Figure 3. Mean ($\pm$ SEM) number of working memory errors (WME) for male and female offspring of high and low LG dams ($n = 6$) on days 1 to 6. Though all groups committed fewer WME as the number of days, on day 3 offspring of low LG dams made more WME than offspring of high LG dams, regardless of sex.

A repeated-measures ANOVA on latency to reach an arm on days 1 to 6 did not find any significant main effects or significant interactions ($.11 \leq p \leq .68$) indicating that as the days progressed the animals did not differ in time taken to reach an arm. There was also no significant effect of litter for latency to reach an arm ($p \geq .60$).

3.3.1. Working memory was significantly enhanced following stress regardless of group, while reference memory was enhanced following stress only in the offspring of low LG dams.

Figure 4 displays the mean ($\pm$ SEM) number of (a) working memory errors (WME), (b) reference memory errors (RME), and (c) working/reference memory errors (W/RME) for pre-stress (day 7) and post-stress (day 9). For WME there was a main

effect of day ($F(1, 20) = 5.91, p \leq .03$) indicating that all groups made fewer WME following exposure to the restraint stress, regardless of group and sex (Fig. 4a). There were no other significant main effects or significant interactions ($.56 \leq p \leq 1.00$). There was also no significant effect of litter for WME ($p \geq .50$).

For RME, there was a strong tendency for an interaction between group (High-LG, Low-LG) and day (pre-stress, post-stress) ($F(1, 20) = 3.52, p \leq .06$; see Figure 4b), with the offspring of low LG dams making fewer RME post-stress but no difference between pre-stress and post-stress performance in the offspring of high LG dams. There was also a strong tendency for a main effect of day for RME ($p \leq .06$). There were no other significant main effects or significant interactions ($.65 \leq p \leq .90$). There was also no significant effect of litter for RME ($p \geq .30$).

For W/RME, there was a significant interaction between day, group, and sex ($F(1, 20) = 5.26, p \leq .03$; see Figure 4c). Post-hoc tests revealed that only the female offspring of low LG dams made significantly fewer W/RME on the post-stress day than on the pre-stress day ($p \leq .02$), however female offspring of low LG dams also made more W/RME than all other groups on the pre-stress day (High-LG males ($p \leq .02$), High-LG females ($p \leq .05$), and Low-LG males ($p \leq .009$)). There were no other significant main effects or significant interactions ($.08 \leq p \leq .77$). There was also no significant effect of litter for W/RME ($p \geq .50$).

A

B

C

Figure 4. Mean ($\pm$ SEM) number of (A) working memory errors (WME), (B) reference memory errors (RME), and (C) working/reference memory errors (W/RME) for male and female offspring of high and low LG dams ($n = 6$) on the day before acute restraint stress (pre-stress day) and the day after acute restraint stress (post-stress day). (a) All groups made significantly fewer WME on the post-stress day than the pre-stress day. (b) There was a trend for offspring of low LG dams to make fewer RME on the post-stress day than the pre-stress day. (c) Female offspring of low LG dams made significantly more W/RME on the pre-stress day than did male offspring of high and low LG dams, and female offspring of high LG dams. Female offspring of low LG dams were the only group to make significantly fewer W/RME on the post-stress day than the pre-stress day.

A repeated-measures ANOVA on latency to reach an arm on pre-stress day and post-stress day showed a significant main effect of sex ($F(1, 20) = 17.81, p \leq .0004$), with female offspring having a shorter latencies to reach an arm than male offspring. There were no other significant main effects or significant interactions ($.09 \leq p \leq .96$). There was also no significant effect of litter for RME ($p \geq .10$).

3.3.2. Proestrus did not account for differences in learning and memory performance.

Analysis of covariance on number of errors with number of days spent in proestrus as a covariate across days did not significantly alter the above findings, as there was a main effect of day ($p \leq .04$) for WME, a tendency for an interaction between group and day ($p \leq .09$) for RME, and only the female offspring of low LG dams made significantly fewer W/RME on the post-stress day than on the pre-stress day ($p \leq .02$). In addition, there was no significant difference in total number of days across testing spent in proestrus between female offspring in the high and low groups ($p \leq .14$).

3.3.3. Corticosterone response to restraint stress appears to be differentially affected by maternal licking and grooming and sex.

A repeated-measures ANOVA with group (High-LG, Low-LG) and sex (male, female) as between-subjects factors and time (baseline, 30 min, 60 min, 120 min) as within-subject factor revealed a significant three-way interaction between time, group, and sex on CORT levels ($F(3, 60) = 3.23, p \leq .03$; see Figure 5). There were also significant main effects of sex ($F(1, 20) = 53.00, p \leq .001$) and time ($F(3, 60) = 70.69, p \leq .001$) and a significant interaction between time and sex ($F(3, 60) = 35.49, p \leq .001$). Post-hoc tests revealed a tendency for male offspring of high LG dams and male offspring of low LG dams (both p 's $< .07$) to have higher baseline CORT levels than female offspring of low LG dams. There were no other significant differences in baseline CORT levels between groups and sex ($.18 \leq p \leq .86$). There was a significant increase between baseline and 30 minute, and 60 minute CORT levels for all groups (all p 's $\leq .04$) except the male offspring of low LG dams ($p \leq .35$). CORT levels significantly increased between 30 and 60 minutes for the female offspring of low LG dams ($p \leq .04$). CORT levels significantly decreased between 60 and 120 minutes for all groups (all p 's $< .05$) except the male offspring of the low LG dams ($p \leq .83$). The CORT levels at 120 minutes remained elevated for female offspring of both high LG dams ($p \leq .0002$) and low LG dams ($p \leq .0001$) compared to baseline, but returned to baseline levels for both male groups ($p > .98$), indicating that the CORT response took longer to recover after exposure to stress in females, regardless of group, than for males. CORT levels in female offspring of high and low LG dams were significantly higher than both male groups at 30, 60 and 120 minutes after the onset of restraint stress (all p 's $\leq .01$). Male offspring of high LG

dams did not significantly differ from male offspring of low LG dams at any time point (all p 's $> .12$). Females of high LG dams had significantly lower CORT levels at 60 minutes compared to female offspring of low LG dams ($p < .05$), but did not differ at other time points (all $p > .20$). There was no significant effect of litter for CORT levels ($p \geq .20$).

Figure 5. Mean ($\pm$ SEM) plasma corticosterone levels of male and female offspring of high and low LG dams ($n = 6$) at baseline, 30, 60, 120 minutes after the onset of acute restraint stress. Female offspring showed a greater corticosterone response to the stress than male offspring. Corticosterone response of female offspring of low LG dams was greater than female offspring of high LG dams at 60 minutes. Corticosterone levels of male offspring of high and low LG dams did not differ at any time point (baseline, 30, 60, 120 minutes). Interestingly, corticosterone levels of all groups, except the male offspring of low LG dams, significantly increased between baseline and 30 minutes after the onset of stress.

3.3.4. Duration of maternal behavior was negatively correlated with initial corticosterone response to acute restraint stress in adult females.

There was a negative correlation between total duration of licking/grooming behavior by dams and the initial corticosterone response to restraint stress of adult female offspring (determined by subtracting baseline CORT levels from the 30 minute CORT levels) ($r = -.58$, $p \leq .05$; Fig. 6), but not in adult male offspring ($p \leq .53$).

Figure 6. The association between duration of licking and grooming behavior of dams and initial corticosterone response (the difference between corticosterone levels at 30 minutes and baseline) to acute restraint stress of male and female offspring. There was a significant negative correlation between duration of licking and grooming and initial corticosterone response in female offspring ($r = -.58$),

indicating that more licking and grooming received by female offspring is coincident with decreased initial corticosterone response to acute restraint stress.

4. DISCUSSION

The results of Experiment 1 show that variation in duration of maternal care enhances working memory of female offspring of low LG dams compared to female offspring of high LG dams. These group differences in behavior were independent of proestrus and latency to reach an arm, indicating that the differences between groups were likely due to mnemonic, and not motor or motivational processes. Furthermore, females required fewer days to reach criterion and committed fewer total errors before reaching criterion than did males. Regardless of sex of offspring, better reference memory was associated with increased levels of licking and grooming by the dam.

The results of Experiment 2 show that variation in duration of maternal care differentially affects the cognitive and neuroendocrine response of male and female offspring to acute restraint stress. All rats showed an enhancement in working memory 24h after acute restraint stress, while only offspring of low LG dams showed an enhancement of reference memory in the spatial working/reference memory version of the radial arm maze after exposure to acute restraint stress. Furthermore, only female offspring of low LG dams showed an acute stress-induced enhancement in working/reference memory, although this is clearly due to the greater number of errors committed by this group on pre-stress day.

Overall, female offspring showed a greater CORT response during acute restraint stress than did male offspring, with female offspring of low LG dams showing the largest CORT response compared to all other groups. Female offspring, regardless of group, also required a longer recovery time after acute stress than did males. Interestingly, all groups showed a significant increase in CORT levels between baseline and 30 minutes and a

decrease in CORT levels between 60 to 120 minutes except male offspring of low LG dams, indicating that males of low LG have an impaired CORT response to stress. These findings are consistent with Pecoraro et al [33] who show phenotypic differences in HPA responses to stress between Sprague-Dawley rats, with some males showing blunted HPA responses. Thus, a sex difference exists in the CORT response to acute restraint stress in offspring of low LG dams, with male offspring of low LG dams experiencing a blunted CORT response compared to both groups of females. Furthermore, increased duration of LG in the dams was associated with decreased CORT response to acute restraint stress in female, but not male, offspring.

Variations in maternal care differentially affect working memory of adult male and female offspring.

In the present study female offspring of low LG dams exhibited enhanced working memory compared to all other offspring. To our knowledge this is the first demonstration that maternal behavior affects memory performance in adult female offspring. Previous research has found that adult male offspring of high, but not low, LG dams show enhanced reference memory performance [2]. Interestingly we did not replicate this finding in males: we found no significant difference in spatial working or reference memory performance between male offspring of high and low LG dams.

The discrepancy between our research and previous findings may be due to strain differences and type of cognitive task used. Sprague-Dawley rats were used in the present experiment, whereas previous research has been conducted on Long-Evans hooded rats. It is possible that Sprague-Dawley rats have different rates of maternal behavior than Long-Evans rats. In the present study we designated dams, and their offspring, as High-

LG or Low-LG if their LG behavior was half a standard deviation above or below the cohort mean. However previous research using Long-Evans rats use a cutoff of one standard deviation [2,22,23,24,34]. Thus in the present study the range in LG behavior in our cohort of Sprague-Dawley dams was not as large as the range in cohorts of Long-Evans rats, possibly indicating why we did not see strong effects of maternal care on cognition or stress reactivity. Regardless, the fact that we saw changes in female performance using these differences in maternal care is intriguing, suggesting that female offspring are more differentially affected by smaller variations in maternal care.

Furthermore, the present study used the working/reference version of the radial arm maze, whereas previous research has investigated spatial reference memory performance using the Morris water maze. Research has shown that these two tasks require different cognitive processes and depend on different forms of motivation (food deprivation versus aversion). Specifically, these two tasks differ in task complexity, search strategies, and task motivation with the radial arm maze being best suited to measure stable asymptotic reference and working memory performance, whereas the Morris water maze measures rapid allocentric spatial reference memory learning (see [35] for review). However, we did find a significant negative correlation between reference memory errors and duration of LG, indicating that increased levels of licking and grooming were associated with better reference memory performance in all offspring of high LG dams, regardless of sex.

Though these findings seem at first glance to be contradictory, it may be that the amount of licking and grooming received by the male offspring of low LG dams may not have been sufficiently low enough to be significantly detrimental to reference memory

performance. It may be that Sprague-Dawley dams do not provide as much maternal care to their offspring as Long-Evans dams (see discussion below) or that the levels of maternal care provided by Sprague-Dawley dams are not sufficiently high enough to be beneficial for spatial learning and memory performance. Menard and Hakvoort [36] suggest that in order for beneficial alterations to offspring development with maternal care, a critical high threshold of maternal care must be surpassed. Thus, the level of maternal LG provided by the high LG Sprague-Dawley dams in the present study may not have exceeded this critical threshold and therefore did not influence the behavior of the male offspring. Furthermore, our present results suggest that the critical threshold for maternal care is different for female and male offspring. Future work should aim to determine the extent of the cognitive differences in female offspring of high and low LG dams, as well as the possible role of strain differences on these effects in male, and potentially female, offspring.

There also may be qualitative rather than quantitative differences in LG that were not addressed in the present study that could account for the differences between our study and past literature. We did not distinguish between overall LG versus anogenital LG and the effects of maternal care may be dependent on this, particularly in male offspring. Moore et al [37] reported that male pups receive more anogenital licking than do female pups. However, it should be noted that past research that has looked at the effects of naturally occurring variations in maternal care on the development of male offspring have combined anogenital and body licking [2,21,22,23,24,25,34]. Future research in this area should consider the possible independent effects of body and anogenital licking.

Acute stress facilitates working memory regardless of sex or variations in maternal care, and improves working/reference memory performance in female offspring of low licking/grooming dams.

Interestingly, in the present study exposure to an acute restraint stress led to an improvement in working memory performance and reference memory performance regardless of sex and duration of maternal behavior. This finding is in partial agreement with past literature that has found enhanced learning and memory performance following acute stress in males [16-18] and in females [1]. However, our findings are not in agreement with past research that has found a sex difference in performance after exposure to acute stress [16-18] or an impairment in working memory performance in males exposed to acute stress [13,28,38]. This discrepancy is likely due to the type of stress and the type of task used. The present study used restraint stress and the spatial working/reference memory version of the radial arm maze to assess learning and memory. Studies that have found a sex difference and/or impairment in memory have used other types of stressors, such as electric shock or cold water restraint, and have also used tasks such as trace eye-blink conditioning or the spatial Y-maze [1,16-18,28]. Working memory is not assessed in either trace eye-blink conditioning or in the spatial Y-maze task and working memory was enhanced following acute stress in the present study.

Variations in maternal care differentially affect corticosterone response of adult male and female offspring to acute restraint stress.

In the present study female offspring showed a greater corticosterone response during acute restraint stress than did male offspring, with female offspring of low LG

dams showing the largest corticosterone response compared to all other groups. Corticosterone levels of male offspring of low and high LG dams did not significantly differ at any time point. Interestingly, this finding is not consistent with previous work that has found that adult male offspring of high LG Long-Evans dams show a reduced CORT response to stress compared to male offspring of low LG Long-Evans dams [2,22,23]. This could be due to differences in the testing paradigm and strain differences as mentioned previously. We exposed Sprague-Dawley offspring to a 120 minute restraint stress and sampled blood throughout that time but not after removal from the restraint, whereas Liu et al [22] exposed Long-Evans male offspring to a 20 minute restraint, sampling blood just prior to the stress, at the 10 minute mark of the stress, and then subsequently after the offspring were removed from the restraint. Therefore, we did not look at recovery from restraint stress in the same manner as Liu et al [22].

In addition in the present study the male offspring had slightly elevated baseline corticosterone levels compared to the females, and the lack of a stress response in the male offspring of low LG dams may have been due to this. At this point, it is not clear why the baseline levels of male offspring of both high and low LG dams were elevated, as blood was collected within 3 minutes of entering the colony room. However, it may be due to the fact that rats underwent food deprivation or that the current study used Sprague-Dawley rats. Food deprivation has been shown previously to upregulate corticosterone levels and this may account for the higher baseline levels seen in the males, although it is unclear why females did not show this same elevation [39,40]. The higher baseline levels may have resulted in a ceiling effect as chronic mild stress can lead to a blunted or reduced CORT response when exposed to an acute restraint stress [41].

Intriguingly, although both male rat groups showed the higher baseline levels, only male rats from high LG dams showed significant stress-induced increases in CORT levels. We used offspring of Sprague-Dawley rats; whereas all other studies looked at the effects of maternal behavior on offspring development in Long-Evans rats [2,22,23,24,34]. It is possible that the effects of maternal behavior on the hypothalamic-pituitary-adrenal responses to stress may be different in Sprague-Dawley rats compared to Long-Evans rats as strain differences in mother-infant interactions have been found previously [37,42]. Long-Evans dams engage in more maternal licking and grooming behaviors than did Fischer 344 dams [37] and a similar relationship with other types of maternal care has been shown between Long-Evans dams and Sprague-Dawley dams [42]. In addition, female offspring of high LG dams responded to the restraint stress with lower levels of corticosterone compared to their low LG counterparts, consistent with previous literature using male rats [2,22,23]. To our knowledge no other work has investigated the effects of maternal care on the neuroendocrine response of adult female offspring to acute restraint stress.

The two present experiments were performed at different times of the day, experiment 1 was conducted in the morning and experiment 2 was conducted in the early afternoon. The endogenous levels of corticosterone follow a circadian rhythm and are at their lowest levels during the light phase, increasing gradually throughout the day [31]. Performance of rats tested in the afternoon may have been influenced by slightly higher corticosterone levels compared to rats tested in the morning. However, research has shown that performance on hippocampal-dependent tasks can be adversely affected by time at which testing occurred in old rats, but not in young rats [43].

Possible neural alterations underlying the effects of maternal care on offspring development.

The effects of maternal LG over the first week postpartum on offspring development seen in the present study and in previous studies may be due to neural changes which are due to changes in gene expression. In particular, the reduced adrenocorticotropin hormone and corticosterone responses to acute stress found in male offspring of high LG dams [22,44] are consequences of increased hippocampal glucocorticoid receptor mRNA and protein levels and decreased hypothalamic corticotrophin release factor mRNA in these animals [22]. Furthermore, cross-fostering the offspring of high and low LG dams reverses the effects of maternal behavior, suggesting a direct relationship between natural occurring variations in maternal behavior and HPA responses to stress [34]. These environmental effects on the response to stress of adult offspring reflect permanent alterations in gene expression. Studies have found that increased levels of pup licking and grooming by Long-Evans dams over the first week of life alters the epigenome at a glucocorticoid receptor gene promoter in the hippocampus of the offspring. Specifically, in adult male offspring of low LG dams there is greater DNA methylation across the glucocorticoid receptor exon 1₇ promoter sequence [44]. DNA methylation is associated with promoting gene silencing through effects on chromatin structure, whereas DNA demethylation, which is believed to occur in offspring of high LG dams at the glucocorticoid receptor exon 1₇ promoter, is associated with gene transcription [45]. Therefore, the demethylated epigenetic status of the glucocorticoid receptor exon 1₇ promoter in the offspring of high LG dams may lead to the dampened stress response in adulthood and the enhanced spatial learning and

memory. However it is important to note that these neural alterations have only been found in male Long-Evans offspring, and may be different in female offspring and different in Sprague-Dawley offspring, thus more research is needed in this area.

5. CONCLUSIONS

The present experiments provide new evidence that variations in maternal care differentially affect hippocampus-dependent spatial working and reference memory performance of male and female offspring. Furthermore, variations in maternal care also affect cognitive and neuroendocrine responses and these effects are dependent on sex, and possibly strain, of the offspring.

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APPENDIX

THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A03-0184

Investigator or Course Director: Liisa Galea

Department: Psychology, Department of

Animals: Rats 391

Start Date: August 1, 2003

Approval Date: June 20, 2006

Funding Sources:

Grant Agency: National Alliance for Research, US (NARSAD)

Grant Title: Models of post-partum depression: Effects on behavior, stress reactivity and hippocampal neurogenesis in both mother and offspring

Grant Agency: Canadian Institutes of Health Research

Grant Title: Parity effects on brain morphology and function

Grant Agency: BC Ministry of Children and Family Development

Grant Title: Morphological changes during pregnancy and the post-partum period relation to mothering experience and behaviours

Grant Agency: BC Ministry of Children and Family Development

Grant Title: Effects of early androgens and corticosterone on stress reactivity and cognition in adulthood

Unfunded title: N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later)