Running head: Reproductive experience, CORT and CBG

Title: Reproductive experience alters corticosterone and CBG levels in the rat dam

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Reproductive experience has significant effects on the brain, behavior and hormone profiles of the mother. Recent work has demonstrated that primiparous rats exhibit decreased dendritic arborizations in the hippocampus, and enhanced hippocampus-dependent spatial memory performance at the time of weaning compared to nulliparous and, to a lesser degree, multiparous rats. Interestingly, enhanced spatial learning and reduced dendritic arbors are seen in nulliparous female rats exposed to chronic stress or repeated corticosterone administration. Based on these observations, we hypothesized that corticosterone may be altered in primiparous compared to multiparous and nulliparous rats. The present study investigated whether the levels of circulating corticosterone and its binding protein, corticosteroid-binding globulin (CBG), are altered with reproductive experience and pup-exposure during late pregnancy and postpartum periods. Total serum corticosterone and CBG were assayed from five groups; multiparous, primiparous, nulliparous, primip-no-pups, and sensitized rats during gestation (days 14 and 19) and the postpartum period (days 1, 5, 14, 21, and 35). Results show that primiparous rats had significantly elevated total corticosterone on postpartum day 1. In addition, primiparous and multiparous rats had significantly lower CBG throughout the postpartum period than all other groups, with primiparous rats exhibiting lower levels than multiparous rats during mid-lactation. These data suggest that free corticosterone is elevated in both primiparous and multiparous dams and is elevated to a greater degree in primiparous compared to multiparous dams during lactation.

Corticosterone and CBG levels were positively correlated with specific maternal behaviors during the first week postpartum in parturient rats, but not in sensitized rats, suggesting a role for corticosterone in the modulation of maternal behavior in parturient rats.

Keywords: corticosteroid binding globulin (CBG), corticosterone, lactation, pregnancy, postpartum, maternal behavior, parity, reproduction, sensitization, steroids

1. Introduction

Pregnancy and motherhood have marked effects on neural, hormonal and behavioral correlates in the mother and these affects appear to differ with reproductive and maternal experience. Recently we found that multiparous females (experienced mothers) do not exhibit the remarkable neural and behavioral changes with motherhood evident in the primiparous female (first-time mother). For example, primiparous rats exhibit decreased dendritic arbors compared to both nulliparous and multiparous females [1] and enhanced spatial memory compared to nulliparous, and to a lesser extent, multiparous females [2] at the time of weaning. Interestingly, enhanced spatial learning coupled with dendritic atrophy in the hippocampus is similar to the pattern seen in chronically stressed nulliparous females [3, 4]. Chronically stressed female rats also exhibit elevated levels of corticosterone and decreased corticosteroid binding globulin (CBG) compared to non-stressed females [3]. Therefore, given our past findings, primiparity, and to a lesser extent, multiparity, may result in high levels of basal corticosterone and/or low levels of CBG during the postpartum period.

Reproductive experience, the number of times pregnant and mothered, has been shown to alter hormone levels. For example, multiparous females have reduced circulating prolactin levels during pregnancy compared to primiparous rats [5], and primiparous females have reduced estradiol and prolactin levels during proestrus compared to nulliparous females [6]. Cortisol, the primary glucocorticoid in humans, is important for a mother's attraction to her infant, particularly in primiparous but not multiparous human mothers [7]. In addition, work investigating the role of glucocorticoid

depletion and administration on maternal behavior in parous females has found that glucocorticoids may be important for retrieval behavior [8, 9]. More importantly, glucocorticoids play a significant role in the maternal experience effect or “maternal memory” [10, 11]. To our knowledge, there is no information about how corticosterone levels may differ between primiparous and multiparous rat dams and how these differences may be associated with maternal behavior. It is possible that a greater elevation in corticosterone may only occur during the first experience with pups and this increase is associated with the solidification of memory for maternal behaviours.

Measurement of plasma corticosterone levels is of limited value if one does not also estimate the binding capacity of corticosteroid binding globulin (CBG) for corticosterone. According to the free hormone hypothesis, globulins are needed to transport steroid hormones to their target tissues but only free steroids (steroids not bound to globulins) are accessible to target tissues [12]. Corticosterone plays an important role in both milk production and maternal memory [10, 13, 14] and both the levels of corticosterone and CBG change remarkably during late pregnancy and lactation in primiparous females [13, 15-21]. It is unknown whether these changes persist with multiparity.

The present work aimed to determine the role of reproductive experience (multiparity, primiparity, and nulliparity) and pup-exposure on corticosterone and CBG levels during late pregnancy, the postpartum period, and post-weaning. In order to investigate these effects, female rats were divided into five age-matched groups: multiparous, primiparous, nulliparous, sensitized and primip-no-pups. We expected

differences in circulating levels of corticosterone and/or a CBG with parity (multiparity vs primiparity vs nulliparity) and we aimed to determine whether any differences were due to the pup-exposure. We also aimed to assess whether corticosterone and/or CBG levels are associated with maternal behavior in the parous and sensitized female during the first week postpartum.

2. Methods

2.1 Animals

Forty-two female Sprague-Dawley rats (*Rattus norvegicus*), approximately 70-80 days of age, obtained from the UBC Animal Care Facility (Vancouver, Canada) were used in the present study. Rats were initially pair housed in opaque polyurethane bins (48 x 27 x 20cm) with aspen chip bedding with Purina rat chow and tap water ad libitum. Rats were maintained in a 12h:12h light/dark cycle (lights on at 7:20 a.m.). All protocols were in accordance with ethical guidelines set by the University of British Columbia Animal Care Committee and the Canada Council for Animal Care.

Rats were randomly assigned to one of five groups: nulliparous (n = 8), primiparous (n = 8), multiparous (n = 9), primip-no-pups (n = 7) and sensitized (n = 7). All animals were age-matched at the time of testing and were approximately 140 days old. Multiparous rats gave birth to their second litter at the same age as primiparous and primip-no-pups rats gave birth to their first litter (140 days). In addition, pup-exposure to females in the sensitized group began when females were the same age as primiparous

dams at the birth of their first litter and multiparous dams at the birth of their second litter. Nulliparous rats were age-matched to these animals.

Day of parturition in parous dams and the first day of pup-exposure to virgins for sensitization (sensitized group) was considered Day 0. Primiparous rats birthed and mothered once. Multiparous rats birthed twice and mothered twice. Nulliparous and sensitized females were virgins. Multiparous and primiparous dams gave birth on Day 0 and had their pups weaned on Day 21 after birth. Sensitized rats were exposed to pups for the same duration of time as primiparous females, from Day 0 to Day 21, and became sensitized during this period (for details see below). Primip-no-pup rats gave birth once on Day 0 and had pups removed permanently within 24 hours of birth. Nulliparous rats that were not exposed to pups or pup-related cues and were singly housed in clear polyurethane cages in a separate room from pregnant and mothering/sensitized rats.

For housing, females in the primiparous, nulliparous, primip-no-pups, and sensitized groups were singly housed at the same time point females in the multiparous group became pregnant with their first pregnancy and were singly housed (see below: Breeding). Females remained singly housed unless paired for breeding (primiparous, primip-no-pups, and multiparous groups). Once pups were removed from any group, females were housed in the same room away from pups and pup-related cues. Nulliparous females were never housed in the same colony room with pups or pregnant females.

2.2 Breeding

For breeding, one female and one male were housed together in a wire mesh cage. Upon release of a vaginal plug females were individually housed in clear polyurethane

bins until birth. For primiparous and multiparous rats, litters were culled to 4 male and 4 female pups approximately 24 hours after parturition. 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). Primiparous rats and nulliparous rats were singly housed in opaque polyurethane bins (48 x 27 x 20cm) in a female-only colony room away from pregnant and lactating rats.

2.3 Maternal Behavior Testing

For maternal behavior observations, the dam and pups were left undisturbed in the home cage. Observations were made every 5 seconds for 10 minutes twice a day (between 8:30-10:00a.m. and 12:00-2:00p.m.) on days 2 to 5 post-parturition. The duration of the following maternal behaviors were observed during each testing period as previously described [22, 23]: licking (body licking and genital licking with the dam off the pups); arched-back nursing (kyphosis); blanket nursing where dam lays over the pups; passive nursing where the dam lies either on her back or side while pups nurse; licking and nursing (dam is on the pups and licking them); and time off pups. Data for each behavior was compiled across all test periods and presented as percent of time spent in a scored behavior. Scores from two multiparous rats are not available.

2.4 Sensitization

Sensitization (pup-exposure in nulliparous rats) was modified from previous research [24] and carried out as previously described [23]. A female was considered sensitized or acting maternal towards pups when pups were licked and hovered over within a 24 hour period as previously described [23, 25]. Briefly, nulliparous rats were continuously housed with 3 donor pups for 22 days starting on Day 0. Three donor pups were used to induce sensitization as this has been found to be sufficient to elicit maternal-type behaviors in nulliparous females [24]. The age of donor pups at the start of sensitization was 5-8 days. The same pups were used throughout the 22 days. Milk-replete pups were given to sensitized rats every 24 hours and the donor pups were returned to their biological mother. Sensitization was confirmed from daily 15 minute video-recordings that took place each morning (between 8am and 11am) immediately after new donor pups were introduced to sensitized rats. Time spent licking pups, hovering over pups (similar posture to arched-back nursing) and sniffing pups was scored from the tapes for the first 7 days of pup-exposure. Behaviors were presented as a percent of time spent in a scored behavior. Spot checks were performed approximately 1, 3 and 24 hours after the video recording to ensure that pups had not been attacked. All video recordings and spot checks took place for the first 10 days of exposure to pups or until sensitization occurred (licked and hovered over pups within a 24 hour period). Pups were removed for that day if a female injured a pup. No female injured pups more than once.

2.5 Blood Collection

Blood was collected from tail nicks; on gestational days 14 and 19, postpartum days 1, 5, 14, 21 (weaning), and 35 in primiparous, multiparous, primip-no-pups rats; at similar time points prior to and during pup-exposure (ie 'postpartum period') in sensitized rats; and at similar time points in nulliparous controls. However, for simplicity, blood collection days in the nulliparous and sensitized rats will be designated using the same day designation as those for parous dams (ie GD14, GD19, PD 1 etc). All blood samples were collected between 7:30-9:00am. Corticosterone levels in the rats have been shown to be elevated within 3 minutes of handling [26], therefore samples were taken within 3 minutes of disturbing the animal (to max 100 μ l). After collection, samples were stored overnight at 4°C and centrifuged at 10,000g for 10 min. Serum was removed from the samples and stored at -20°C.

2.6 Corticosterone Assays

All samples were run in duplicate using a commercially available RIA kit for rat corticosterone from MP Biomedicals (Orangeburg, NY, USA). The average intra-assay coefficient of variation for all assays was below 10%. The standard curve ED80 was between 15.5 and 32.3 ng/ml, ED50 was between 114.5 and 161.8 ng/ml, and for ED20 was between 710.9 and 927.0 ng/ml, with a detection limit of 7.7 ng/ml.

2.7 Corticosteroid binding capacity

Corticosteroid binding capacity, which reflects the level of CBG, was determined following procedures developed by Hammond and Lähteenmäki [27]. Briefly, plasma samples were diluted 1/1000 and incubated 30 minutes at room temperature with dextran-coated charcoal (DCC) suspension to remove endogenous steroids. The dextran-coated

charcoal was then precipitated by centrifugation and an aliquot of the samples was added into duplicate tubes containing 1 picomole of [1,2-³H] corticosterone (specific activity: 50Ci/mmol; ARC, St Louis, MO, USA) and to 1 tube containing 0.5 μ M of cold corticosterone for the evaluation of the non-specific binding. After incubation for 1 hour at room temperature, tubes were placed into an ice-water bath for 30 minutes. Ice-cold DCC was then added to remove unbound steroids and the reaction was incubated for another 10 minutes and centrifuged at 2800 rpm at 4°C for 10 minutes. The incubation with the cold DCC requires a correction factor (off rate) in the calculation of the binding capacity to account for the dissociation during DCC separation. This correction was determined experimentally and estimated at 80%. Supernatants were transferred into scintillation vials and 4 ml of Aqueous Counting Scintillant (Amersham Bioscience, UK) was added. The samples were counted in a scintillation spectrophotometer Beckman Coulter LS6000K. Specifically-bound counts were obtained by subtracting the non-specific background counts to the average of the total bound counts and converted in pmol/ml of serum. Interassay variability was 6.2%.

2.8 Data Analyses

Separate repeated-measures analysis of variance (ANOVA) tests were calculated on basal corticosterone and CBG with group (nulliparous, primiparous, multiparous, primip-no-pups, sensitized) as the between-subjects variable and day (GD14, 19, PD1, 5, 14, 21, 35) as the within-subjects variables. To determine differences in litter characteristics, ANOVAs were performed on litter size, number of male, and number of female pups and group (primip-no-pups, primiparous and multiparous). To determine

differences in duration of maternal care in parous females, a repeated-measures ANOVA was performed between duration of a maternal behavior (licking, arched-back nursing, etc) and group (primiparous and multiparous). Pearson product-moment correlations were conducted between percent of time spent in specific maternal behaviors, day of onset of 'maternal behavior' in sensitized rats, with corticosterone levels and CBG from GD14 to PD5. Post-hoc comparisons utilized the Fisher LSD test.

3. Results

Litter size, sex of offspring and duration of maternal care was not significantly different between parous rats. A one-way ANOVA revealed no significant difference between primiparous, multiparous, and primip-no-pups rats in the size of the litter at birth, the number of male or female pups (all $.1 \leq p \leq .6$; data not shown). A repeated-measures ANOVA revealed no significant main effect of group (primiparous vs. multiparous; $p \leq .4$) or an interaction effect between group and type of maternal behavior ($p \leq .2$). Post-hoc tests revealed that dams spent significantly more time blanket nursing than all other maternal behaviors (all p 's $\leq .001$; main effect of maternal behavior ($F(4, 52)=37.5, p < .001$; Table 1). Further differences are described in Table 1.

Total corticosterone was significantly affected by parity. A repeated-measures ANOVA on total serum corticosterone level revealed a significant interaction effect of group by day ($F(24, 204) = 1.9, p \leq .01$; Figure 1). Post-hoc tests revealed that primiparous rats had significantly elevated corticosterone on PD1 compared to

multiparous, primip-no-pups, and sensitized rats (all p 's $\leq .02$). Multiparous rats had significantly elevated corticosterone on PD35 compared to primip-no-pups ($p \leq .04$), nulliparous rats had significantly elevated corticosterone on GD19, PD14, and PD35 compared to all other groups ($.003 \leq p \leq .01$), and on PD1 compared to multiparous, primip-no-pups and sensitized rats (p 's $\leq .005$). A one-way ANOVA comparing corticosterone levels in sensitized females at the time points females were individually sensitized (ranging from 2-8 days) with corticosterone levels on PD 1 in parous females revealed primiparous females have significantly elevated circulating corticosterone levels on PD1 compared to the sensitized, multiparous, and primip-no-pup females (all p 's $\leq .001$; $F(3, 27) = 8.56$, $p \leq .001$). There were no other significant differences between groups ($.08 \leq p \leq 1.0$). There was also a significant main effect of group ($F(4, 34) = 6.5$, $p \leq .001$) and day ($F(6, 204) = 7.1$, $p \leq .001$).

CBG was significantly decreased in lactating dams and was significantly lower in primiparous dams than multiparous dams mid lactation. A repeated-measures ANOVA on CBG revealed a significant interaction effect of group by day ($F(24, 204) = 8.8$, $p \leq .001$; Figure 2). Post-hoc tests revealed that primiparous rats had significantly lower CBG than multiparous rats on PD 14 ($p \leq .02$) and PD 35 ($p \leq .02$), and tended to have lower CBG than multiparous rats on PD 5 ($p \leq .07$). In addition, primiparous, multiparous and primip-no-pups rats had significantly lower levels of CBG than nulliparous and sensitized rats on GD 19 (p 's $\leq .03$) and PD1 (p 's $\leq .04$), primiparous and multiparous rats had significantly lower levels of CBG than all other groups on PD5 (p 's

$\leq .001$), PD14 (p 's $\leq .001$) and PD21 (p 's $\leq .001$), and primiparous rats also had significantly lower levels of CBG than nulliparous rats on PD35 ($p \leq .02$). There were no other significant differences between groups (all p 's $> .1$). There was also a significant main effect of group ($F(4, 34)=5.9$, $p \leq .01$) and day ($F(6, 204) = 22.1$, $p \leq .001$).

Corticosterone and CBG correlated with specific maternal behaviors. There were significant positive correlations between percent of time spent licking by parturient dams in the first week postpartum and total serum corticosterone levels of PD1 ($r = .58$, $p \leq .02$; Figure 3a), percent of time spent blanket nursing and CBG on GD19 ($r = .61$, $p \leq .015$; Figure 3b), as well as percent of time in the nest (total time licking and nursing) and CBG on GD19 ($r = .55$, $p \leq .03$; Figure 3c). There were no other significant correlations between specific maternal behaviors and CBG or corticosterone levels between GD14 and PD5 ($p > .1$). Sensitized rats took an average of 4.1 ± 0.9 days (range 2 to 8 days) of pup exposure before being considered maternal (licked and hovered over pups within a 24 hour period). There were no significant correlations between levels of corticosterone or CBG and day of onset of sensitized maternal behavior ($.3 \leq p \leq 1.0$) or for any maternal behaviors for sensitized rats before and after sensitization ($.1 \leq p \leq .9$).

4. Discussion

The results demonstrate that reproductive experience affects corticosterone and CBG levels during the postpartum period. Here we show that primiparous rats have significantly elevated total basal corticosterone on first postnatal day compared to

multiparous, primip-no-pups, and sensitized rats. In addition, CBG levels decreased in lactating dams (both primiparous and multiparous rats) and this decrease in CBG was dependent on reproductive experience with primiparous rats having lower levels than multiparous rats mid lactation (PD 5 and 14) and after weaning (PD 35). These results are not due to differences in degree of maternal care or particular litter characteristics between the parous rats. Elevated levels of total basal corticosterone on postpartum day 1 were also associated with more time spent licking offspring, and elevated levels of CBG on gestation day 19 were associated with greater time blanket nursing offspring and greater time spent in the nest (total time licking and nursing) in parous, but not sensitized, females. The results also demonstrate that nulliparous rats had significantly elevated levels of total basal corticosterone on GD19, PD 14 and PD35 compared to other groups.

The finding that multiparous female dams do not show the same elevation in corticosterone on PD1 as primiparous females is consistent with previous work in humans [28]. Multiparous women show a weaker elevation in circulation corticosterone levels as primiparous women during the early postpartum period [28]. Thus first-time motherhood may be linked to higher levels of corticosterone compared to second-time motherhood in both humans and rodents. Lactating primiparous rats are reported to have elevated basal corticosterone levels throughout the postpartum period when compared to nulliparous females [21, 29]. This is partially consistent with the results from the present study (Figure 1). However, others report either no significant difference or an overall decrease in peripheral corticosterone levels during mid lactation compared to nulliparous rats [19, 30-32]. Discrepancies between previous findings and the present results may be

due to the method of blood collection, control groups used (nulliparous or parous without pups), and time of day tested. For example, research demonstrating that corticosterone levels are elevated during lactation in lactating dams analyzed corticosterone levels from trunk blood obtained through decapitation and compared them to nulliparous females or parous females without pup exposure [17, 21, 29]. Whereas research demonstrating an elevation in corticosterone levels in primiparous rats on postpartum day 1 and not later in the postpartum period took blood samples from the tail nicks and compared them to nulliparous females [present work and [32]. It is possible that these different techniques could result in differences in the level of circulating corticosterone measured as research in other species has found the level of steroids in the blood is dependent on the blood withdrawal site (carotid artery versus jugular vein; [33]).

Peripheral corticosterone is particularly important in the lactating dam for milk production and maternal memory [10, 13, 34]. However, circulating levels of corticosterone may be very different than neural levels. Previous work has demonstrated that corticosterone levels are significantly elevated in the hippocampus, cerebral cortex, and hypothalamus of lactating dams compared to nulliparous females on postpartum days 14-15, even when peripheral corticosterone levels did not differ between groups [31]. Thus, differences in neural levels of corticosterone are evident at different time points than in the periphery. Regardless, it is certainly well documented that elevated levels of peripheral circulating corticosterone and cortisol, due to corticosterone administration or in response to stress and disease, have numerous neural effects [3, 35-37].

The role of corticosterone in maternal behavior in parturient females.

Our findings of increased total basal corticosterone levels on the first postpartum day in primiparous rats and positive correlations between corticosterone or CBG levels and maternal behaviors in parous rats is consistent with recent research demonstrating the importance of glucocorticoids in maternal memory particularly in the first-time mother [10]. In the human, first-time mothers with high levels of cortisol were more attracted to their infant [38]. In the rodent, adrenalectomized primiparous rats show lower levels of maternal behaviors such as licking [9] and corticosterone administration to adrenalectomised primiparous females reduces the latency to act maternal after the re-introduction of pups [10]. In agreement with Rees et al. (2004), we found that basal corticosterone levels on the first postpartum day in parturient females are positively correlated with licking during the early postpartum period in dams. Additionally we found that CBG levels during *late gestation* are positively correlated with blanket nursing during the early postpartum period and time spent in the nest. It is perhaps not surprising that gestational levels of CBG would relate to postpartum behavior, as previous work has demonstrated that gestational levels of hormones may contribute to feelings of attachment in women; in women low estradiol-to-progesterone ratios during late pregnancy are associated with high feelings of attachment to one's infant during the early postpartum period [7].

Basal corticosterone levels in nulliparous females exposed to pups.

In the present study we found nulliparous females exposed to pups and pup-related cues, who became sensitized within 4 days of direct pup-exposure, did not have the same elevated levels of corticosterone as nulliparous females not exposed to pups. Interestingly, previous work has demonstrated that sensitized nulliparous females exposed to pups show a decreased corticosterone response [39]. Therefore, it appears that pup-exposure or the activity of maternal behavior in sensitized females acts to decrease or perhaps stabilize corticosterone levels. Whether this is in part due to changes in the regularity of the estrous cycle has yet to be determined [40]. We did not assess stage of estrous cycle throughout testing to confirm these effects.

In the present study females in the sensitized group were not fully maternal at the beginning of pup-exposure and it is well documented that nulliparous females take much longer to become maternal compared to parturient females [41]. Thus it is possible that corticosterone levels may have differed if sensitized females were used from the beginning of the postpartum period. We did not investigate whether there was an increase in basal levels of corticosterone in the sensitized group upon initial introduction to pups (Day 0) and it is possible corticosterone levels were elevated on this day. Previous research has demonstrated that pup-exposure to naïve virgin females results in elevations in corticosterone levels on the first day of exposure to pups but that once sensitized, introduction of pups results in a decrease in circulating corticosterone [39]. Consistent with Koranyi et al (1977), our data demonstrates there was a slight elevation in basal corticosterone levels in females in the sensitized group on PD1, 24 hours after initial pup-

exposure, and once sensitization occurred (mean of 4 days of pup-exposure) basal corticosterone and CBG levels either were lower or did not change from PD1 (see Figures 1 and 2). For example when comparing corticosterone and CBG levels on PD5 of sensitized rats (one day after sensitization actually occurred) with levels on PD1 of primiparous and multiparous rats it is clear that primiparous dams have higher levels of basal corticosterone on PD1 compared to sensitized rats on both postpartum days 1 and 5. Therefore, it is possible that a greater elevation in corticosterone during the early postpartum period in primiparous dams, but not multiparous dams or sensitized females, is needed to permanently alter neural circuitry, develop long-term maternal memory, facilitate milk production and maintain adequate maternal behavior.

Unlike parous females, there were no significant correlations between corticosterone or CBG levels with the onset of ‘maternal behavior’ (licking and hovering within a 24h period) or specific maternal behaviors (sniffing, hovering, licking) in intact nulliparous females exposed to pups (sensitized females) in the present study. Previous research has demonstrated that adrenalectomized nulliparous females show increased licking and time with pups compared to adrenalectomised nulliparous females administered corticosterone and sham controls [42]. This, coupled with our present results, suggests that the relationship between circulation corticosterone and maternal behaviors is reversed or altered in non-parturient females.

It is also possible that the number of pups used for sensitization may have affected the levels of corticosterone and quality and quantity of maternal behavior. Sensitized females in the present study were exposed to three foster pups, which is sufficient to

sensitize virgin females [23, 24], however the parous females in the present study were exposed to 8 pups. Past research has used between 3 and 8 pups for sensitization [25, 41, 42], but to our knowledge how the number of foster pups affects maternal behavior and hormone levels in sensitized females has not been investigated. It is possible that exposure to more pups may alter the maternal responses and corticosterone levels of nulliparous females during sensitization.

Different criterion for the onset of maternal behavior may have also affected the present results. For example, we did not specifically quantify pup retrieval in our experiment [42]. Regardless, half of our sensitized females retrieved during video recordings. Reasons for not using the retrieval test were to provide an environment consistent with that of parous females who were left undisturbed, and to not disturb the nest site which may, in itself, cause an artificial elevation in corticosterone levels.

The decrease in CBG in lactating dams is affected by reproductive experience.

Consistent with previous research, we found that CBG is decreased in lactating dams [13-16]. In the present study, multiparous females did not exhibit the same level of decrease in serum CBG levels as primiparous females during lactation (PD5 and PD14) and after weaning (PD 35). The differences in CBG between multiparous and primiparous dams during the early postpartum period further support the idea of the predominant role for corticosterone in the induction of maternal memory with primiparity. In addition, these changes may be needed to facilitate milk production in primiparous dams. This is important because a decrease in circulating CBG levels will

cause a transient increase in free corticosterone levels which are needed for both the formation of maternal memory and milk production [10, 13, 43]. Interestingly, decreased CBG levels persist in primiparous dams after weaning which suggests a long term alteration in CBG in primiparous females that may contribute to the differences we see in hippocampus structure and function with reproductive experience [1, 23, 25, 29, 32]. The duration of these effects have yet to be determined, but they do not appear to persist to the same degree with additional reproductive experience (multiparous), as demonstrated in the present study.

Our results also indicate that the decrease in CBG levels in parturient females during lactation is dependent on processes associated with lactation and not pup-exposure. We found that parturient females with pups removed within 24 hours of birth (primip-no-pups) and pup-exposed virgin females (sensitized) did not show a decrease in CBG levels. In addition, the CBG levels in parous dams increase after weaning and differences are no longer observed between multiparous rats and non-parous rats. Previous work has demonstrated the size of the litter affects corticosteroid binding capacity with a greater decrease in corticosteroid binding capacity in dams with larger litters [15], thus providing more evidence that lactation and suckling is important for the decrease in CBG levels in the dam. In the present study it should be noted that effects between primiparous and multiparous dams were not due to litter size as all litters were culled during the early postpartum period. Taken together these data confirm that lactation and suckling is involved in the alteration of circulating CBG levels.

Although the mechanism behind the decrease in CBG levels during lactation has yet to be determined, it seems plausible that the thyroid hormone, thyroxine (T4), and glucocorticoids may play a role. T4 has been shown to significantly increase CBG biosynthesis in the male rat, probably through an increase of mRNA stability [44]. Interestingly, T4 shows a dramatic decrease during lactation in rat dams which is associated with increased lactation intensity or suckling burden [45, 46]. A decrease in T4 levels may contribute to decreased CBG during lactation. Furthermore, high levels of glucocorticoids have been shown to feedback and decrease CBG levels in female rats [47]. Therefore high levels of corticosterone, as seen in the present study in primiparous rats, may result in a further decrease in CBG during lactation. Prolactin and oxytocin also play a major role during lactation [13, 39, 48] and given that CBG levels are decreased with lactation it is possible that these peptides may be involved in the control of CBG.

Conclusions

Results from the present study demonstrate that reproductive experience differentially affects serum corticosterone and CBG levels in the dam. Here we show a pronounced increase in basal corticosterone levels on the first day postpartum and the decrease in CBG levels throughout lactation that is greater in primiparous females compared to multiparous females. Our results demonstrate that parity has significant effects on circulating levels of corticosterone and may be particularly important for the initial transition to motherhood.

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Table 1. Mean ($\pm$ SEM) percent of time during observations that primiparous and multiparous dams spent performing specific maternal behaviors. There was no significant differences between primiparous and multiparous females in maternal behavior ($p \leq .4$) or an interaction effect between group and type of maternal behavior ($p \leq .2$).

Behavior (%)	Primiparous	Multiparous
Lick	1.5 ± 0.6	0.6 ± 0.3
Lick and Nurse	14.8 ± 3.6	10.9 ± 2.3
Arched Nurse	25.5 ± 4.1	14.3 ± 4.5
Blanket Nurse	49.7 ± 6.4	55.2 ± 6.9
Passive Nurse	3.8 ± 1.6	12.3 ± 5.3

Figure Legends

Figure 1. Mean ($\pm$ SEM) total serum corticosterone (nM). Primiparous rats had significantly elevated levels of corticosterone on PD1 compared to multiparous, primip-no-pups, and sensitized rats (all p 's $\leq .02$), multiparous rats had significantly elevated levels of corticosterone on PD35 compared to primip-no-pups ($p \leq .04$), and nulliparous rats had significantly elevated levels of corticosterone on GD19 (p 's $\leq .001$), PD14 (p 's $\leq .01$), and PD35 (p 's $\leq .001$) compared to all other groups, and on PD1 compared to multiparous, primip-no-pups and sensitized rats (p 's $\leq .005$). *denotes primiparous significantly different from multiparous, primip-no-pups and sensitized rats; # denotes multiparous significantly different from primip-no-pups. Mean of nulliparous females given.

Figure 2. Mean ($\pm$ SEM) serum corticosteroid binding globulin (CBG)(nM). Primiparous rats had significantly lower CBG than multiparous rats on PD 14 ($p \leq .02$) and PD 35 ($p \leq .02$), and tended to have lower CBG than multiparous rats on PD 5 ($p \leq .07$). In addition, primiparous, multiparous and primip-no-pups rats had significantly lower levels of CBG than nulliparous and sensitized rats on GD 19 (p 's $\leq .03$) and PD1 (p 's $\leq .04$), primiparous and multiparous rats had significantly lower levels of CBG than all other groups on PD5, PD14 and PD21 (p 's $\leq .001$), and primiparous rats also had significantly lower levels of CBG than nulliparous rats on PD35 ($p \leq .02$). * denotes primiparous rats significantly different from multiparous rats; # denotes primiparous, multiparous and primip-no-pups significantly different from nulliparous and sensitized; + denotes

primiparous and multiparous rats significantly different from all other groups; @ denotes primiparous rats significantly different from nulliparous rats.

Figure 3. Correlations between total serum corticosterone or CBG levels and maternal behavior.

There was a significant positive correlation between (a) percent of time spent licking in the first week postpartum and total serum corticosterone levels on PD1 ($r = .58, p \leq .02$), (b) percent of time spent blanket nursing and CBG level on GD19 ($r = .61, p \leq .015$) and (c) percent of time in the nest (total time licking and nursing) and CBG level on GD19 ($r = .55, p \leq .03$). ○ = primiparous, ● = multiparous.

Figure 1

Figure 2

Figure 3