Chronic corticosterone during pregnancy and postpartum affects maternal care, cell proliferation and depressive-like behavior in the dam

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Abstract

Stress during pregnancy and the postpartum can influence the well-being of both the mother and her offspring. Prolonged elevated levels of glucocorticoids are associated with depression and we developed an animal model of postpartum depression/stress based on high levels of corticosterone (CORT) during the postpartum. Gestational stress is a risk factor for postpartum depression and prenatal and/or postnatal high levels of CORT may have differential effects on the mother. Thus the present study was conducted to investigate the effects of low (10mg/kg) or high levels of CORT (40mg/kg) given to dams either during gestation, postpartum or across both gestation and postpartum on maternal care, depressive-like behavior and hippocampal cell proliferation in the dam. Only the high dose of CORT administered during the postpartum increased depressive-like behavior in the dam. Furthermore the high dose of CORT altered maternal care (reduced time spent on the nest and nursing) regardless of whether administration of CORT was during gestation or postpartum. Gestational and/or postpartum treatment with high CORT and postpartum low CORT reduced cell proliferation in the dentate gyrus of postpartum dams compared to oil-treated controls. Thus prolonged treatment with high levels of CORT postpartum reduced maternal care, hippocampal cell proliferation and induced depressive-like behavior in the dam and therefore might be considered an animal model of postpartum depression. More research is needed to understand the effects of stress hormones during different phases of reproduction and how they affect the brain and behavior of the mother and her offspring.

Keywords: stress; gestation; rat; mood disorders; glucocorticoids; neurogenesis; forced swim test; depression; postpartum depression; antenatal depression

Introduction

Pregnancy and the postpartum period are associated with hormone fluctuations, changes in neuroendocrine function and regulation. For instance, the sensitivity and regulation of the hypothalamic pituitary adrenal (HPA) axis is altered during pregnancy and the postpartum in both humans and rodents (Brunton and Russell, 2008). This altered sensitivity, possibly initiated through pregnancy-related steroid hormones and peptides, such as oxytocin, is believed to play a role in damping stress reactivity during this time (Lightman et al., 2001; Torner et al., 2002), and likely protects the offspring from the potential harmful impact of elevated glucocorticoids.

Despite this dampening of the stress response during pregnancy and the postpartum, stress during these times can have long lasting effects on the outcome of the offspring in both humans and rodents (Maccari and Morley-Fletcher, 2007; Brummelte and Galea, 2010b). For example chronic stress during pregnancy reduces the amount of maternal licking and reduced licking or touching of the infant can affect developmental outcomes in both rodents and primates (Liu et al., 1997; Fleming et al., 1997; Nomura et al., 2002). Furthermore exposure to chronic stress is cited as a preceding factor for depression (Parker et al., 2003) and maternal depression during pregnancy and/or the postpartum affects not only the well-being of the mother but also the development of the child (Murray and Cooper, 1997; Halligan et al., 2007).

It is well known that chronic stress can affect behavior, emotion, cognition and brain morphology (Galea et al., 1997; Sousa et al., 2000; McEwen, 2008). For instance, chronic stress increases anxiety-like and depressive-like behaviors (D'Aquila et al., 1994), impairs learning and memory (Bowman et al., 2003) and suppresses neurogenesis in the hippocampus (Mirescu and Gould, 2006) of male rodents. However, stressed females often show a different effect than males, for example chronic restraint stress impairs spatial memory in males but enhances spatial memory in females (Bowman et al., 2003; Westenbroek et al., 2004; Shors et al., 2007).

Therefore, it has been suggested that females exhibit a different vulnerability towards stress or elevated glucocorticoids compared to males (Dalla et al., 2005). However, less is known about how pregnant or lactating females react to chronic stressors or high levels of glucocorticoids (Leuner et al., 2007).

Chronic stress is cited as the preceding factor in 85% of cases of depression and a dysregulation of the HPA axis is a common finding in patients with depressive symptoms (Jolley et al., 2007; Binder et al., 2009; Schule et al., 2009). It is clear that there is a significant link between stress, elevated glucocorticoid levels and depression (Nestler et al., 2002; McIsaac and Young, 2009) and stress during pregnancy or the postpartum period can in turn contribute to gestational or postpartum depression (Nestler et al., 2002; Misdrahi et al., 2005; Brummelte and Galea, 2010b). However little research has focused on the question of how stress or elevated glucocorticoids during pregnancy or postpartum can affect the mother's themselves (Brummelte and Galea, 2010b).

In fact the childbearing years are considered the time of greatest risk for women to develop major depression (Kessler, 2003; Drevets and Todd, 2005; Marcus and Heringhausen, 2009). Approximately 15 % of new mothers develop postpartum depression (Kornstein et al., 2002; Goodman, 2007), while the reported prevalence of perinatal depression varies from 10-23% (Gaynes et al., 2005; Gorman, 2006). Hormonal fluctuations, including the changes in cortisol, are believed to play an important role in the establishment of depressive symptoms during pregnancy or the postpartum (Brummelte and Galea, 2010b). For instance, the normal diurnal rhythm of pulse secretion induced by the HPA axis is lost during lactation, such that mean basal levels remain constant throughout the day (Lightman et al., 2001), a phenomenon which is also observed in depressed patients (Parker et al., 2003; den Hartog et al., 2003; Schule et al., 2009).

Animal models of depression often use chronic stressors (Konkle et al., 2003; Herzog et al., 2009) and/or high levels of corticosterone (CORT) to induce depressive-like symptomology in male and female rats (Kalynchuk et al., 2004; Gregus et al., 2005). Chronic high CORT administered to adult male and female rats leads to an increase in depressive-like behavior in a dose- and sex-dependent manner (Hill et al., 2003; Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006). Recently we adapted this animal model of depression to induce postpartum depressive-like symptoms in rat dams (Brummelte et al., 2006). Using the administration of high levels of CORT instead of a physical or social stressor allows for more controllability of the hormone levels in the dams, less individual variability, avoids maternal separation and/or exposing the offspring to the same stressor. We found that chronic corticosterone injections (40mg/kg) to the dams postpartum led an increase in depressive-like behavior (immobility in the forced swim test), a reduction in body weight, and reduced maternal care (more time off the nest and less time nursing) in the dams. Taken together, chronic high corticosterone postpartum may serve as a potential valuable animal model of postpartum depression as it mimics several symptoms or findings for the human disorder such as increased despair, body weight changes and reduced maternal care (Lovejoy et al., 2000; Faulconbridge et al., 2009).

One major risk factor for developing postpartum depression is antenatal depression or gestational stress (Milgrom et al., 2008). Stress during gestation can increase depressive-like behavior in the postpartum period and alter maternal care in rodents (Smith et al., 2004; Misdrahi et al., 2005; O'Mahony et al., 2006). However, to our knowledge, the effect of direct corticosterone administration during gestation on maternal care and depressive-like behavior postpartum has not been investigated.

The hippocampus is particularly vulnerable to the effects of stress and elevated glucocorticoids due to the high concentration of glucocorticoid receptors (Meaney et al., 1985;

McEwen, 1999). The hippocampus retains the ability to produce new neurons in adulthood in most mammalian species (Gould, 2007). Hippocampal volume and adult hippocampal neurogenesis are reduced in depressed patients (McKinnon et al., 2009; Boldrini et al., 2009) and in animal models of depression (Vollmayr et al., 2007; Jayatissa et al., 2008; Green and Galea, 2008). In addition, chronic stress and/or high corticosterone suppresses hippocampal neurogenesis in both male and female rats (Pham et al., 2003; Wong and Herbert, 2006; Brummelte and Galea, 2010a). Thus in the current study we wanted to examine cell proliferation in the hippocampus, a putative neural marker of depression, in our animal model of PPD/stress.

The present study was conducted to investigate the effects of two doses of CORT (10mg/kg; 40mg/kg), administered during gestation, the postpartum or both, on maternal behavior, depressive-like behavior and hippocampal cell proliferation in the postpartum. In the present study dams received daily injections of either oil or CORT from gestational day 10-20 and/or from postnatal day 2-24. At the end of the postpartum period, dams were tested in the forced swim test (depressive-like behavior) and open field test (anxiety-like behavior) and their brains processed for markers of cell proliferation in the hippocampus. We hypothesized that high levels of CORT administered during the postpartum period or during pregnancy and the postpartum period will increase immobility in the forced swim test and reduce maternal care and cell proliferation. Further, we expected the dams to be somewhat protected from harmful effects of the lower dose of CORT if it is administered only during pregnancy or the postpartum but a stronger effect if it administered during both periods.

Materials and Methods

Animals

Forty-six female Sprague–Dawley rats at 3 months of age were obtained from the University of British Columbia Animal Care Facility (Vancouver, Canada) and were used in this study. Rats were initially housed in pairs in opaque polyurethane bins ($48 \times 27 \times 20$ cm) with absorbent bedding and were given Purina rat chow and tap water ad libitum. Animals were maintained on a 12 h:12 h light/dark cycle (lights on at 7:30 a.m.). In addition, 20 sexually experienced males (approx 4-5 month old) were used for mating. 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.

Procedures

Figure 1 gives an overview of the experimental procedures. For breeding, one female and one male were paired in a wire mesh cage and upon release of a vaginal plug (1-5 days) females were individually housed in polyurethane bins. All dams were handled for 4 days prior to injections except for the animals from the undisturbed group (control; $n=5$), which were left alone during the whole time except for cage changing and weighing. On day 10 of pregnancy the remaining rats were then randomly assigned to one of the following groups with daily s.c. injections of combinations of either oil, low CORT (10mg/kg) or high CORT (40mg/kg). These groups consisted of: Oil controls ($n=10$) received sesame oil injections (1ml/kg) during pregnancy and postpartum; Preg10 ($n=5$): received low CORT dose during pregnancy and oil injections during postpartum; Post10 ($n=5$): received sesame oil during pregnancy and low CORT during the post partum period, P+P 10 ($n=5$): received low dose of CORT during pregnancy and the postpartum, Preg40 ($n=5$) received high CORT during pregnancy and oil during postpartum, Post40 ($n=6$): received oil during pregnancy and high CORT during the post partum period and P+P 40 ($n=5$)

received high CORT during pregnancy and the postpartum. For an overview over the groups see Table 1. Subcutaneous injections were performed daily around 10 a.m. from day 10-20 of pregnancy and day 2-24 of postpartum, thus allowing the animal to be undisturbed during parturition. One day after birth (birth day = day 0), all litters were culled to 5 males and 5 females per dam. Maternal care observations were done from PPD 2-8 (see below). After completion of the maternal care observations, dams and their litters were moved to maternal bins (48 x 40 x 27). Pups were weaned on day 23 and used in a different study. Four hours after their last CORT or oil injection on postpartum day 24 dams received an injection of bromodeoxyuridine (BrdU; 200mg/kg), a DNA synthesis marker, and were perfused 24 hours later to examine cell proliferation in the dentate gyrus of the hippocampus (Taupin, 2007).

Hormone Preparation

An emulsion of corticosterone (CORT; Sigma-Aldrich, St. Louis, MO; USA) was prepared every few days at a concentration of 10mg/ml (low dose) or 40 mg/ml (high dose) by mixing CORT with 10% ethanol in sesame oil. The high dose was chosen because it causes elevated blood levels of CORT for a prolonged period of time (Sapolsky, 1985; Johnson et al., 2006), induced depressive-like behavior in males and females (Kalynchuk et al., 2004) and reduced cell proliferation and adult neurogenesis in the hippocampus of both males and females (Wong and Herbert, 2006; Brummelte and Galea, 2010a). The low dose was chosen because it does not alter depressive-like behavior or baseline CORT levels in male rats, but it diminishes their CORT-response to stress (Johnson et al., 2006).

Measures

Body weight and litter characteristics

Body weight was measured on day 7, 10, 14 and 20 of pregnancy and approximately every 4 days (day 1, 9, 13, 17 and 20) during the postpartum. Due to high individual differences in dams' body weight before and after birth, body weight gain or loss for the dams was calculated and analyzed as percent change in body weight over time. We calculated body weight gain or loss during gestation by setting the weight on the day of the 1st CORT injection (GD10) as 100% and calculating the percentage gain or loss for body weight on GD20. Body weight gain or loss during the postpartum was calculated accordingly by setting the weight on the PD1 as 100% and calculating the percentage gain or loss for body weight on PD20.

Length of pregnancy was estimated as time between the day sperm was observed and the time of day when giving birth (morning, afternoon or night). The number of male or female pups were counted and weighed on postnatal day 1 before litters were culled to 5 males and 5 females. The average weight per pup was calculated by dividing the litter weight by the number of pups per litter. If there were not enough males or females in one litter, pups were either cross-fostered from a dam within the same group who gave birth the same day (3 cases) or if there were no pups available for cross-fostering when the litter was culled (2 cases), the dam kept an uneven number of male and female pups.

Serum corticosterone levels

Serum samples were taken on postpartum day 23 from each dam at 8 a.m. within 3 min of touching the cage to examine basal levels of CORT and to verify elevated serum CORT in the postpartum CORT groups. Samples were stored overnight at 4°C, centrifuged at $10 \times g$ for 10 min and serum stored at -20°C until further processing. Total CORT was measured using the ImmuChem Double Antibody 125-I radioimmunoassay kit (MP Biomedicals, Orangeburg, NY,

USA); with an intra-assay and inter assay coefficient of variation less than 7.5% and a sensitivity of 7.7ng/ml. All reagents were halved and samples run in duplicates.

Behavioral Measures

Maternal behavior

On postpartum days 2 to 8 maternal behavior was assessed as previously described (Pawluski et al., 2006). The duration of the following maternal behaviors in the dams were observed during each testing period: licking (body licking and genital licking with the dam off the pups); licking/nursing; arched-back nursing; “blanket” nursing; passive nursing; and time off pups. Scoring of behavior occurred twice a day for 10 min with test times being randomly chosen during the light cycle, but with at least 2 h between the sessions and testing occurred no earlier than 2 h after injections. Data were then aggregated across days and calculated as percent time spent in each behavior.

Forced swim test

The forced swim test is a widely used test for antidepressant efficacy (Porsolt et al., 1978) that has also been more recently used as a test for depressive-like behavior (Galea et al., 2001; Smith et al., 2004; Dalla et al., 2005; Hinojosa et al., 2006). This test was used to assess depressive-like behavior in the dams at the end of the postpartum period. The test was used as previously described (Galea et al., 2001). Briefly, the apparatus consisted of a vertical cylindrical glass container (45 × 28 cm) filled to a depth of 30 cm with tap water at $25 \pm 0.5^{\circ}\text{C}$. Session 1 of the forced swim test took place 2 hours after the CORT or control injection on postpartum day 21 and lasted 15 min. The 2nd session of the test followed 24 hours later and dams were then placed

in the cylinder for 10 min. All test sessions were videotaped and the 2nd session was later scored by an observer blind to conditions. The behaviors scored in the forced swim test were: (1) struggling—quick movements of the forelimbs such that the front paws break the surface of the water; (2) swimming—movement of forelimbs or hind limbs in a paddling fashion; and (3) immobility—floating in the water with only movements necessary to keep the head above water.

Open field test

The open field test (OFT) was used to measure locomotor activity (Wilson et al., 1976) and exploratory behaviors (Crusio, 2001). The apparatus, a 120×120 cm² arena divided into 16 squares of equal dimension with 40 cm high walls, was placed in a dimly lit room, with a video camera installed in the center above the apparatus. On postpartum day 23 dams were placed in the field facing a corner and activity was video recorded for a 10 min period. An observer blind to conditions scored the number of squares, peripheral or central, entered by the animals (an entry was defined as all four paws in a quadrant), the number of rears and the number of fecal boli from each dam. In addition, the videos were analyzed with ‘Anymaze’ (Stoelting Co., Wood Dale, USA), a software for automatic behavioral scoring to assess the total distance travelled by the animal.

Immunohistochemistry

In the afternoon of postpartum day 24 all dams received a single i.p. injection of Bromodeoxyuridine (BrdU; 200mg/kg), a DNA synthesis marker. Twenty four hours later rats were deeply anesthetized with sodium pentobarbital and then perfused with saline followed by 4% paraformaldehyde. Extracted brains were stored in 4% paraformaldehyde for 24 h, and then transferred into a solution of phosphate-buffered sucrose solution (30%) for a minimum 48 h.

Brains were sliced through the entire extent of the dentate gyrus using a vibratome (Leica VT1000S) at 40 µm thickness. Sections were collected in 0.1M phosphate buffered saline (0.9%; PBS) and stained for BrdU to measure cell proliferation.

BrdU

BrdU immunohistochemistry was performed as described previously (Epp et al., 2007). If not otherwise stated tissue was rinsed three times in TBS (0.1 M Tris–phosphate buffer in 0.9% saline; pH 7.4) between each step. Briefly, sections were first rinsed, then treated with 2 N HCl for 30 min at 37°C, followed directly by one rinse in 0.1M borate buffer for 10 min, three rinses in PBS and then incubation in 3% normal horse serum. Tissue was then directly transferred into the primary antibody solution containing 1:200 mouse BrdU antibody (Roche, Mississauga, ON; Canada), 3% normal horse serum (NHS) and 0.1% Triton-X for 48 hours. Sections were then incubated in anti-mouse secondary antibody (1:200; Vector Laboratories, Burlington, ON, Canada) for 4 h, followed by incubation in ABC solution (Vector Laboratories, Burlington, ON, Canada) for 2h. Then sections were developed for approximately 5 min in 0.02% diaminobenzidine (DAB; Sigma Aldrich Chemicals) with 0.0003% H₂O₂, mounted on slides, allowed to dry overnight, lightly counterstained with cresyl violet acetate, dehydrated and coverslipped with Permount (Fisher Scientific, Nepean, CDA).

Cell counting

Counting was performed by an experimenter blind to the animal's treatment. Cells were counted in every 10th section throughout the entire granule cell layer (GCL) and subgranular zone (SGZ) of one hemisphere using a Nikon microscope at 1000x magnification. Cells in the GCL + SGZ were counted separately from cells in the hilus to account for potential changes in the blood-brain

barrier permeability. The area of the dentate gyrus was measured using an image analysis software (ImageJ, NIH) and the total volume for dentate gyrus was estimated using Cavalieri's principle (Gundersen and Jensen, 1987). The total number of BrdU-labeled cells was estimated by multiplying the total number counted by 20 as previously described (Kronenberg et al., 2003; Eadie et al., 2005; Spritzer and Galea, 2007) and total density was calculated by dividing total number of cells by the volume of the dentate gyrus.

Data analyses

If not otherwise stated, data was analyzed using a repeated-measures analysis of variance (ANOVA). Weight gain during pregnancy was analyzed with an analysis of covariance (ANCOVA) with treatment during pregnancy (Control (n=5), Oil (n=20), Preg10 (n=10), Preg40 (n=11); see table 1) as the between-subjects factor and with the litter size as the cofactor. Total weight gain postpartum was analyzed with a one-way ANOVA with group (Control, Oil, Preg10, Post 10, P+P10, Preg40, Post40, P+P40) as between-subjects factor. Separate repeated-measures ANOVAs were calculated for maternal behavior (total time spent nursing, licking and grooming and time off nest) and nursing posture (passive, blanket, ABN) as the within-subjects factors and group (Control, Oil, Preg10, Post 10, P+P10, Preg40, Post40, P+P40) as the between-subjects factors. The open field test was analyzed by a repeated-measures ANOVA with group as the between-subjects factor and number of crossings or distance travelled (peripheral, center) as the within-subjects factors. Number of rearings, time spent immobile and number of fecal boli were also analyzed. Forced swim test behavior was analyzed using repeated measures ANOVA with behavior (struggling, swimming, immobile) as within-subject factors and group as the between-subjects factor. For serum CORT levels taken on postnatal day 23 a one-way ANOVA was performed with group as the between-subjects factor. Density of proliferating cells and volume of

the dentate gyrus were analyzed with a repeated-measures ANOVA with area (hilus, GCL) as within-subjects factors and group as between-subjects factors. All post-hoc tests utilized LSD.

Results

Body weight

An ANCOVA, using litter size as the covariate, on the percent gained between day 10 and 20 of pregnancy revealed a significant main effect of group ($F(3, 39)=20.68, p<.001$), with no effect for the covariate litter size ($p=.10$). Post-hoc tests revealed that dams who received CORT during pregnancy gained less weight compared to oil-treated or control dams (Preg10: $p=.002$ vs. control dams and $p=.03$ vs. Oil dams; Preg40: both p 's $<.001$; Figure 2A).

Weight change postpartum (day 1-20) was analyzed using a one-way ANOVA. Results showed a significant main effect of group ($F(7, 37)=10.32, p<.001$). Post-hoc tests revealed that Post10, Post40 and P+P40 showed attenuated weight gain, with the latter two groups showing weight loss during the postpartum compared to weight gain in control or oil dams (Post10: $p=.05$ and $.01$ vs. control and oil respectively, Post40 and P+P40: all p 's $<.001$). There were no significant differences in weight gain in any other groups (all p 's $>.09$; Figure 2B)

Pregnancy length and litter characteristics

There was no significant difference between prenatal treatment groups (control, oil, Preg10, Preg40; cc table 1) for the length of pregnancy ($p=.09$), but there was a significance difference in litter size between prenatal treatment groups ($F(3, 41)=2.78, p=.05$) and litter weight ($F(3, 41)=5.69, p=.002$). Post-hoc tests showed that dams in the Preg40 group had fewer pups

compared to undisturbed control dams ($p=.03$), but not compared to oil-treated dams ($p=.13$). Further, Preg40 litters weighed significantly less compared to any other group ($p<.03$), however an analysis of covariance showed that litter size had a highly significant effect on the litter weight ($p<0.001$). Further, there was no significant difference in average pup body weight ($p=.20$) between the groups, suggesting that the reduced litter weight in Preg40 was due to the smaller litter size. There was no significant difference between treatment groups in the number of males and females ($p=.98$) or number of dead pups ($p=.62$). Litter characteristic data are presented in table 2.

Maternal care

There was a significant interaction of group and type of maternal behavior ($F(14, 76)=6.55$, $p<.0001$). Post hoc tests revealed that the P+P10, Preg40, Post40 and P+P40 groups spend more time off the nest (all $p's<.01$) and less time nursing (all $p's<.05$) than oil controls (Fig.3). There was no significant difference in amount of time spent licking for any of the groups (all $p's>.71$). Further, the different types of nursing postures (blanket, passive, arched back nursing (ABN)) were analyzed with a repeated measures ANOVA. There was a significant main effect of group ($F(7, 38)=7.15$, $p<.0001$) and type of nursing ($F(2, 76)=44.8$, $p<.0001$) and a trend for a significant interaction ($F(14, 76)=1.75$, $p=.063$). Post-hoc tests showed that Post10, P+P10, Preg40, Post40 and P+P40 groups spend less time nursing compared to controls (all $p's<.05$) and ABN was more frequent than blanket, and blanket more frequent than passive nursing for all groups (all $p's<.0001$; Figure 3).

High CORT during the postpartum increased immobility in the forced swim test

Due to the heterogeneity of variance the data were transformed with the arcsin transformation. The ANOVA revealed a significant interaction effect of group and behavior ($F(14, 68)=4.95$, $p<.0001$), and a main effect of behavior ($F(2, 68)=215.6$; $p<0.0001$), but no main effect of group ($p=0.77$). Post-hoc tests showed that the Post40 group spent more time immobile and less time swimming compared to all other groups (all p 's < 0.02). In addition P+P10, Preg40 and P+P40 groups spent more time immobile compared to undisturbed controls ($p=.03$, $.04$ and $.03$ respectively) but not compared to oil controls (all p 's $> .13$) (Figure 4). There were no other significant differences between any groups on the amount of time spent struggling, swimming or immobile.

There were no group differences in Open Field Test behavior.

The ANOVA for number of peripheral or central crossings revealed neither significant group effect nor an interaction effect (p 's $> .54$). There were also no significant differences between groups on the number of rearings ($p=.37$), distance travelled ($p=.35$), number of fecal boli ($p=.28$) or total/central crossings ratio ($p=.13$; table 3) between groups.

High postpartum CORT groups had higher levels of serum CORT than all other groups.

Not surprisingly, there was a significant treatment effect on dam's CORT levels on postpartum day 23 ($F(7, 34)=20.988$, $p<.0001$). Post-hoc tests showed that high CORT during the postpartum period (Post40 and P+P40) causes substantially elevated serum CORT levels compared to all other groups (p 's $< .0001$), while the low CORT postpartum had no significant effect on CORT levels postpartum (Fig.5).

There is no significant difference in dentate gyrus volume between groups.

A repeated measures ANOVA revealed a significant effect of area ($F(1,31)=682.9$; $p<0.001$) with a greater volume of the hilus than the granule cell layer but no main effect of group ($p=0.5$) or interaction effect ($p=0.85$). Data are presented in table 4.

High and low CORT postpartum reduced cell proliferation in the dentate gyrus

A repeated measures ANOVA revealed a significant effect of group ($F(7,31)=2.63$; $p=0.03$), a significant effect of area ($F(1,31)=222.81$, $p<0.001$) and a significant interaction effect ($F(7,31)=2.23$; $p=0.05$). Post hoc tests showed that the Post10, Preg40, Post40 and P+P40 dams had significantly reduced density of BrdU-labeled cells in the GCL + SGZ compared to oil controls ($0.05>p>0.001$; Figure 6). Furthermore the density of BrdU-labeled cells in the GCL + SGZ were significantly different between the undisturbed controls and all CORT-treated groups ($0.03>p>0.0001$), but not between the oil and undisturbed controls ($p=0.11$).

Discussion

The results from the present study demonstrate that exposure to exogenous corticosterone during gestation or the postpartum leads to dose-dependent and time of administration-dependent changes in body weight, maternal care, forced swim test behavior and cell proliferation in the dentate gyrus but does not affect open field test performance. We show that low or high dose CORT treatment during pregnancy or the postpartum attenuated body weight gain in the dams, and CORT during pregnancy reduced litter size. Further we showed that dams exposed to low CORT during pregnancy and the postpartum or exposed to high CORT at any time point showed reduced maternal care (more time off the nest and less time nursing) with no difference in licking compared to oil controls. Interestingly only the Post40 group expressed depressive-like behavior

in the forced swim test. Furthermore gestational and/or postpartum treatment with high CORT or postpartum low CORT reduced cell proliferation in the dentate gyrus of dams. Thus, as expected, the high dose of CORT had a stronger effect on behavioral and neuronal markers of depression in the dams but only dams given high CORT during the postpartum displayed high face validity with postpartum depressive symptoms.

CORT treatment during pregnancy or the postpartum attenuates body weight gain in the dams and high CORT during pregnancy reduced litter size

As expected, all dams gained a significant amount of weight during pregnancy. We found that dams treated with CORT during pregnancy gained less weight between day 10 and 20 of pregnancy compared to controls, which is in line with previous studies showing reduced weight gain during pregnancy with glucocorticoid or stress exposure in rats (Mairesse et al., 2007; Igosheva et al., 2007; Franko et al., 2010). The observed attenuated weight gain during the postpartum period in dams treated with high or low CORT is in line with previous studies in postpartum or virgin rats (Brummelte et al., 2006; Johnson et al., 2006). In humans weight loss is associated with depressive symptoms and weight change and appetite disturbances are listed as single compound symptoms of depression by the DSM IV.

It is important to note that dams in our Preg40 group had a significantly lower number of pups compared to unhandled controls, but not compared to oil controls. However, an ANCOVA showed no significant effect of litter size on maternal body weight, thus the reduced weight gain was likely not due to difference in litter size. The reduced litter size in the Preg40 group is consistent with studies showing that prenatal stress or dexamethasone treatment can affect litter

weight (O'Regan et al., 2004; Zagron and Weinstock, 2006; Mairesse et al., 2007; Franko et al., 2010).

CORT reduces maternal care in a dose- and time –dependent manner

In the current study we found that dams that received either high CORT during gestation or postpartum or low CORT during both pregnancy and the postpartum spent significantly less time on the nest and nursing than controls. These results are in line with our previous study showing that high CORT during the postpartum reduces maternal care (Brummelte et al., 2006) and other studies showing negative association between high glucocorticoid levels and maternal care (Bahr et al., 1998; Bardi et al., 2003). Further Kurata et al (2009) showed that postpartum dams who exhibited more depressive-like behavior showed less active nursing compared to postpartum dams who exhibited less depressive-like behavior.

Only high CORT or prolonged exposure to low CORT were effective in reducing maternal care. In line with this, a very low dose of CORT administered to dams through the drinking water either leads to an increase (Casolini et al., 2007) or no change in maternal licking (Casolini et al., 1997) and a more positive outcome of the offspring, while high dose CORT during the postpartum leads to a more negative outcome of the offspring (Brummelte et al., 2006).

There is substantial evidence from both animal and human studies that maternal care can affect the outcome of the offspring (Gunnar, 1998; Meaney, 2001; Barha et al., 2007). It is believed that increased maternal licking during early postnatal development can counteract detrimental effects of pre- or postnatal environmental stress on the offspring (Casolini et al., 2007; Del Cerro et al., 2010), but stress during gestation can also decrease maternal care, including reduced maternal licking (Smith et al., 2004). Therefore it is crucial to understand

factors that alter maternal behaviors in order to ensure the well-being of the mother and the offspring.

High levels of CORT during the postpartum increases depressive-like behavior only in postpartum dams

Consistent with previous results (Brummelte et al., 2006) our current data shows that high CORT during the postpartum results in more immobility and reduced swimming in the forced swim test compared to oil controls. Our findings are further in line with previous studies on virgin male and female rats showing that high CORT induces depressive-like behavior (Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006). Chronic treatment of male rats with 20mg/kg, but not 10mg/kg, of CORT induced depressive-like behavior (Hill et al., 2003; Johnson et al., 2006). Interestingly, prolonged exposure to the high dose of CORT (P+P40) did not significantly increase immobility in the forced swim test unlike with the shorter exposure time (Post 40). This apparent discrepancy might be due to altered HPA axis activation in the P+P40 group due to an extensive exposure to high levels of CORT including gestation or to the ability of female rats to adapt to the prolonged treatment with CORT.

The fact that we did not see any significant differences in the open field test suggests that the difference in the forced swim test is not due to general changes in locomotor activity in the CORT treated animals.

CORT administration during pregnancy and/or the postpartum reduces hippocampal cell proliferation

Elevated levels of corticosterone reduced hippocampal neurogenesis and cell proliferation in male and virgin female rodents (Wong and Herbert, 2006; Brummelte and Galea, 2010a).

Further, motherhood and reproductive experience affects hippocampal neurogenesis as primiparous rats show lower levels of cell proliferation and survival during the postpartum period compared to virgin females (Leuner et al., 2007; Pawluski and Galea, 2007). However, to our knowledge, this is the first study to show that chronic treatment with corticosterone during pregnancy and/or the postpartum can also effectively downregulate hippocampal cell proliferation in the postpartum dam. Interestingly, treatment with high CORT during pregnancy also reduces hippocampal cell proliferation on postpartum day 25 despite the long period (~4 weeks) between the last CORT injection and the time of assessment. This suggests that high CORT exhibits long lasting effects on the hippocampal neurogenic activity consistent with evidence in males (Heine et al., 2004).

Interestingly CORT decreased cell proliferation in all high dose groups and the Post10 group, but only the Post40 group showed more depressive-like behavior in the forced swim test. Thus in the present study we found a dissociation between reduced hippocampal cell proliferation and depressive-like behavior in postpartum females. This dissociation is consistent with other studies (Bessa et al., 2008), including another animal model of postpartum depression from our laboratory (Green and Galea, 2008) but inconsistent with a number of other studies showing a link between depressive like behavior in the forced swim test and hippocampal cell proliferation (Llorens-Martin et al., 2007; Veena et al., 2009; Zhang et al., 2010). Thus while there are often cases where depressive-like behavior coincides with reduced cell proliferation (as seen for the Post40 group in the present study) it does not necessarily follow that the two processes are always related (Airan et al., 2007; Bessa et al., 2008).

Unique HPA axis function and glucocorticoid sensitivity in females during pregnancy and the postpartum

Pregnancy and the postpartum period are times of suppressed HPA axis function and reduced glucocorticoid and stress sensitivity (Johnstone et al., 2000; Russell et al., 2001; Douglas et al., 2003; Brunton and Russell, 2008). However, CORT levels in rats and cortisol levels in humans are naturally increased during pregnancy and the postpartum (Abou-Saleh et al., 1999; Leuner et al., 2007; Pawluski et al., 2009; Yim et al., 2009), while at the same time the increase of HPA activity normally seen in response to either physical or psychological stresses in the non-reproductive state become severely attenuated or absent during lactation (Windle et al., 1997a; Windle et al., 1997b; Lightman et al., 2001; Tu et al., 2005). This is due to hormone-induced changes in the brain and HPA axis in preparation for motherhood such as reduced CORT release after adrenocorticotrophic hormone (ACTH) stimulation and reduced corticotropin-releasing factor expression in the paraventricular nucleus (Johnstone et al., 2000). In addition, CORT decreases ACTH secretion more slowly in pregnancy, indicating a decrease in rapid feedback sensitivity of the HPA axis (Johnstone et al., 2000). This altered glucocorticoid sensitivity and feedback mechanism is in line with our observation, that serum values of CORT in the CORT postpartum females in the present study compared to virgin females given the same dose for 21 consecutive days (Brummelte and Galea, 2010a) is approximately half as high (~400 vs. 800 ng/ml after high dose; 50ng/ml vs. 200 after low dose). This suggests that there is a greater adaptation to increased CORT levels in dams during the postpartum. However it should be noted that the high dose of CORT given in the present study during pregnancy or the postpartum is still potent enough to chronically increase CORT levels and will likely disturb HPA axis function despite the downregulation of the HPA axis during the postpartum.

Another important observation from our results is that high levels of glucocorticoids during the postpartum period are more damaging to the dam than high levels of glucocorticoids during gestation. This might be due to different mechanisms of HPA axis attenuation during late

pregnancy and the postpartum rendering the postpartum dam more vulnerable to the damaging effects of elevated glucocorticoids (Douglas et al., 2003; Brunton et al., 2008). For example during late pregnancy ACTH and CORT show a flattened circadian rhythm, while in the postpartum period CORT and ACTH show an elevated basal secretion due to changes in the oxytocin and vasopressin system (Brunton et al., 2008; Slattery and Neumann, 2008). Thus adaptations like the changes in ACTH and CORT from late pregnancy to the postpartum make the rat dam less resistant to stress compared to pregnant dams. Thus the postpartum is a critical time in an adult female's life during which external glucocorticoid manipulations might have a unique impact on HPA axis and neurological function. However, it is also important to note that dams treated with CORT during pregnancy had more time to recover compared to dams treated during the postpartum as most testing took place at the end of the postpartum period. More studies are needed to understand the unique impact of stress during pregnancy versus stress during the postpartum period on the maternal and infant health.

High levels of CORT as a potential animal model of postpartum depression

Despite the high prevalence of postpartum depression in mothers, the etiology of this disease and the potential consequences for the development of the children are not well known. Animal models might help to better comprehend the complex relationship between the effects of glucocorticoids and effects on maternal mood and offspring development. Pregnancy and postpartum are associated with complex changes in HPA system which lead to sustained high flattened levels of glucocorticoids in both the human (Magiakou et al., 1996; Brunton et al., 2008) and the rodent (Lightman et al., 2001; Pawluski et al., 2009). This is a similar profile observed in depressed patients, suggesting that pregnancy and the postpartum period may be a time of increased vulnerability to depression in susceptible women. In the present study we found that high

postpartum CORT reduced body weight, maternal care and hippocampal cell proliferation compared to oil controls, indicating that this may be a potential model of postpartum depression.

Pregnant females or dams treated with CORT showed an attenuated weight gain in our study. In humans and rodents, weight loss or attenuated weight gain are associated with depressive symptoms (Konkle et al., 2003; Johnson et al., 2006; Chaput et al., 2008; Faulconbridge et al., 2009) and weight change and appetite disturbances are listed as single compound symptoms of depression by the DSM IV. Further we found that high corticosterone during gestation or the postpartum reduced maternal care by less time spent nursing and on the nest. Reduced maternal care is often observed in depressed human mothers (Atkinson et al., 2000; Lovejoy et al., 2000) as depressed mothers are less positive towards their infants (Field, 1998), touch their infants less (Ferber, 2004) and show poorer mother-infant interaction and attachment (Atkinson et al., 2000; Lovejoy et al., 2000). Furthermore mothers that suffer from postnatal depression breastfeed for a shorter duration than control mothers (Halligan et al., 2004; Groer and Morgan, 2007). Thus our finding of reduced maternal care with high CORT levels during pregnancy or the early postpartum reflects symptoms seen in depressed mothers.

High CORT during gestation, postpartum and prolonged during both gestation and postpartum reduced cell proliferation in the hippocampus in the present study. Reduced neurogenesis and cell proliferation have been found in several animal models of depression (Vollmayr et al., 2007; Jayatissa et al., 2008; Green and Galea, 2008) and treatment with antidepressants can restore neurogenesis to normal levels (Malberg et al., 2000; Banasr et al., 2006; Jaako-Movits et al., 2006; Jayatissa et al., 2008), an effect which depends on adrenal steroids (Wong and Herbert, 2004; Huang and Herbert, 2006). Interestingly however, only high CORT during the postpartum period lead to increased immobility in the forced swim test in the dams compared to oil controls. Thus, taken together, our results show that show that high levels

of CORT during the postpartum (Post40) decreases body weight, maternal care and hippocampal cell proliferation and increases depressive-like behavior thus displaying high face validity with symptoms of depression in human mothers. Therefore our results strengthen the idea of using high CORT during the postpartum as an animal model of postpartum depression.

Undisturbed versus oil controls: the case of two controls

In the present study we had two control groups: one was left undisturbed throughout the experiment and was only handled for changing cages and weighing, while the other group received daily sesame oil injections to account for the daily handling stress of administering CORT injections. Although we did not find significant differences between our undisturbed and oil controls for any of the investigated measures, for some measures treatment groups differed significantly from the undisturbed controls, but not from the oil controls. This suggests that daily handling (as would have been experienced by the oil controls) and/or s.c. injections might have a slight effect on the dam's behavioral and neurological outcome. It is well known that neonatal handling can have a profound effect on the developmental outcome (Meaney et al., 1989; e.g. Padoin et al., 2001) and our data underlines the importance of proper control groups for prolonged daily handling even in adulthood.

Conclusion

Taken together, the results show that the chronic administration of a high dose of CORT, especially during the postpartum period, might serve as a valuable model to study postpartum depression. Treatment with high levels of CORT during the postpartum period led to increased depressive-like behavior in the forced swim test, reduced weight gain, reduced maternal care and

suppressed cell proliferation in the hippocampus of postpartum dams. With the exemption of the forced swim test behavior the prolonged treatment with CORT during pregnancy and the postpartum seemed to have an amplifying effect on behavior and cell proliferation. Gestational and postpartum depression are considered among the most under-recognized and diagnosed diseases in women (Marcus, 2009) and more research is needed to understand the complex relationship between stress hormones and maternal and infant health during this critical time. Our animal model might help to better comprehend the cellular and behavioral changes in mothers suffering from postpartum depression and thus help to improve the conditions and well-being of the mother and the child.

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Fig 1. Experiment Overview

Fig. 2: Body weight change during pregnancy and the postpartum

A: Dams who receive CORT during pregnancy (low or high dose) gain significantly less body weight during the time of treatment (gestational day 10-20). B: High levels of CORT (Post40, P+P40) during the postpartum period cause significant body weight loss in dams postpartum compared to undisturbed control or oil dams. Treatment with low levels of CORT during the postpartum period (Post10), but not during pregnancy and the postpartum (P+P10), leads to significantly less weight gain compared to oil-treated or undisturbed controls; * $p \leq .05$; *** $p \leq .001$ vs. undisturbed control and oil dams

Fig. 3 Maternal care

Dams who received the high dose of CORT or the low dose for a prolonged period of time (i.e. P+P10) showed reduced time on the nest (A) and time nursing (B). There was no significant difference between the groups regarding the time they spent licking their pups (C); a = significantly different from undistributed control dams, b= significantly different from oil dams

Fig. 4 Dams' Forced Swim Test

A high dose of CORT during the postpartum period (Post40) resulted in less time spent swimming (B) and more time spent immobile (C) in the forced swim test (session 2). There was no significant difference in the time spent struggling (A) or any other differences between groups; a = significantly different from undisturbed control dams, b= significantly different from oil dams

Fig 5: Dams' corticosterone concentration on Postpartum day 23 (sample taken at 8am in the morning thus approx. 24hrs after CORT injection). Dams treated with the high dose of CORT during the postpartum period (i.e. Post40 and P+P40) showed significantly higher CORT levels compared to any other group; *** $p < .001$

Fig. 6

The density of BrdU-labeled cells in the granule cell layer (GCL) + subgranular zone (SGZ) and hilus of the dentate gyrus, 24 hours after BrdU injection. All CORT treated dams had lower levels of cell proliferation than undisturbed controls and all high CORT groups and the Post10 group had lower levels than oil controls; a = significantly different from undisturbed control dams, b= significantly different from oil dams.

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Table 1

Table 1: Treatment groups

GROUP NAME	TREATMENT DURING PREGNANCY (DAY 10-20)	TREATMENT DURING POSTPARTUM (DAY 2-24)
Control (n=5)	-	-
Oil (n=10)	Oil	Oil
Preg10 (n=5)	10mg/kg CORT	Oil
Post10 (n=5)	Oil	10mg/kg CORT
P+P10 (n=5)	10mg/kg CORT	10mg/kg CORT
Preg40 (n=5)	40mg/kg CORT	Oil
Post40 (n=6)	Oil	40mg/kg CORT
P+P 40 (n=5)	40mg/kg CORT	40mg/kg CORT

Table 2: Litter characteristics

	UNDISTURBED (n=5)	OIL (n=20)	PREG10 (n=10)	PREG40 (n=11)
Length of pregnancy [d]	21 $\pm$ 0	20.93 $\pm$.09	21.2 $\pm$.11	20.8 $\pm$.08
Litter size	17.2 $\pm$.66	15.25 $\pm$.59	16.8 $\pm$.57	14.1 $\pm$.94 ^a
Body weight per pup (PD1)	6.91 $\pm$.08	6.68 $\pm$.15	6.73 $\pm$.12	6.28 $\pm$.23
Males (n)	8.2 $\pm$.80	7.5 $\pm$.50	8.1 $\pm$.70	7.0 $\pm$.69
Females (n)	9.0 $\pm$.31	7.75 $\pm$.62	8.7 $\pm$.79	7.1 $\pm$.67
Dead pups (total n per group)	0	3	0	3

There was no significant difference between the groups regarding length of pregnancy, pups' body weight, number of males or females or the number of dead pups. There was however a smaller litter size for PREG40 dams. PD: postnatal day; a = $p < .05$ compared to undisturbed control dams

Table 3. Open Field Test

	UNDISTURBED	OIL	PREG1	POST1	P+P10	PREG4	POST4	P+P40
			0	0		0	0	
peripheral crossings	106 ± 9	103 ± 8	110 ± 14	106 ± 10	112 ± 6	111 ± 5	105 ± 5	135 ± 18
central crossings	12 ± 2	8 ± 4	9 ± 3	15 ± 5	16 ± 2	19 ± 4	14 ± 6	17 ± 7
total/central crossing ratio	28.75 ± 9.15	11.33 ± 1.70	24.85 ± 11.40	15.0 ± 7.41	8.51 ± 0.98	8.74 ± 1.95	8.20 ± 1.37	24.56 ± 10.82
total distance travelled [m]	47 ± 3	43 ± 3	51 ± 6	38 ± 9	42 ± 9	50 ± 3	46 ± 3	59 ± 7
#of rearings	53 ± 4	46 ± 4	49 ± 6	50 ± 4	49 ± 2	57 ± 6	61 ± 3	54 ± 4
# of fecal boli	1.9 ± .8	2 ± .8	3.3 ± 1.1	2.8 ± .9	3.6 ± 1.5	0.6 ± .6	1.2 ± .6	0.8 ± .8

There was no significant difference between the groups for any of the measures.

Table 4: Volume of the dentate gyrus

	UNDISTURBED	OIL	PREG10	POST10	P+P10	PREG40	POST40	P+P40
Granule Cell Layer (GCL) Area	2.10 ± 0.15	1.93 ± 0.08	2.06 ± 0.21	1.95 ± 0.10	2.43 ± 0.12	2.19 ± 0.07	2.12 ± 0.16	2.05 ± 0.21
Hilus Area***	4.93 ± 0.12	5.08 ± 0.19	5.20 ± 0.5	4.77 ± 0.42	5.63 ± 0.39	5.48 ± 0.59	5.06 ± 0.33	4.68 ± 0.35

There was no significant difference between any of the groups in the volume of the granule cell layer (GCL) or the hilus. However, there was a significant effect of area (*** $p < 0.001$), with the hilus having a greater volume than the GCL.

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