Gestational and postpartum corticosterone exposure to the dam affects behavioral and endocrine outcome of the offspring in a sexually-dimorphic manner

Susanne Brummelte[1+], Stephanie Lieblich[1] and Liisa A.M. Galea[1,2]*

[1] Department of Psychology and [2]Brain Research Center, University of British Columbia, Vancouver, BC, Canada,

*Corresponding author: Liisa A.M. Galea, Ph.D., Professor, The University of British Columbia, 2136 West Mall, Vancouver, B.C. V6T 1Z4, Phone: (604) 822-6536, Fax: (604) 822-6923, Email: lgalea@psych.ubc.ca.

[+]Present Address: Developmental Neurosciences & Child Health, Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada

Abstract

Exposure to high levels of glucocorticoids in utero and during the postpartum period has a detrimental effect on brain development. We created an animal model of postpartum stress/depression based on administering high levels of corticosterone (CORT) to the dams during the postpartum period which caused behavioral changes and reduced hippocampal cell proliferation in the offspring. As the consequences of early exposure to glucocorticoids may depend on the dose and the developmental stage of the offspring, 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 the outcome of the offspring. Male and female offspring were weighed throughout the experiment, tested in a series of behavioral tests (forced swim test, open field, elevated plus maze) and basal and restraint stress CORT levels were examined in adolescence or young adulthood. Results show that maternal CORT exposure, regardless of when administered, significantly attenuated body weight gain until adulthood in the offspring. Offspring exposed to low maternal CORT, but not high maternal CORT, during the postpartum had higher basal levels of CORT as young adults. Further, male and female offspring of dams exposed to high maternal CORT *in utero* showed more depressive-like behavior in the forced swim test, while males of dams exposed to high maternal CORT postpartum exhibited more anxiety-like behavior in the elevated plus maze. Taken together, maternal glucocorticoid exposure have long lasting effects on male and female offspring's behavioral and neuroendocrine measures in adolescence and adulthood depending on the time of exposure to glucocorticoids.

Keywords: gestation, prenatal, *in utero*, glucocorticoids, depression, stress, sex differences, early programming, development, adolescence

1. Introduction

Stress during pre- or postnatal development can have a profound impact on the long term outcome of the individual. The maternal hypothalamus pituitary adrenal (HPA) axis is downregulated during pregnancy, partly to protect the offspring *in utero* from harmful effects of elevated glucocorticoids (Brunton et al., 2008). However, despite the alteration of the HPA axis function of the mother, prenatal stress profoundly affects the neurodevelopment of the offspring (Talge et al., 2007). For instance, if a rat dam is exposed to psychological stress during her pregnancy, her male offspring display depressive-like behaviors in the forced swim test and altered HPA axis function compared to offspring from a non-stressed dam (Abe et al., 2007). Similarly, male and female children born to women who experienced stress during pregnancy had a higher risk for developing depressive symptoms and severe depression as young adults compared to age-matched controls from non-stressed mothers (Watson et al., 1999). Further, exposing the pregnant dam to restraint stress causes changes in anxiety, spatial learning, neuroendocrine function and receptor levels in the brain of the offspring, which are highly sex-dependent (Darnaudery and Maccari, 2008; Zuena et al., 2008). It is also well known that early postpartum stress, such as maternal separation, can have profound and sex-dependent effects on the adult phenotype of the F1 (e.g. Gross et al., 2010; Renard et al., 2007; Slotten et al., 2006), and F2 offspring generations (Franklin et al., 2010). In addition early stress can affect maternal behavior in the lactating female offspring (Bosch et al., 2007) as prenatal and postnatal exposure to stress also changes maternal behavior in the dam. For instance, chronic stress during pregnancy reduces maternal licking (Smith et al., 2004), while high maternal corticosterone (CORT) treatment postpartum reduces time spent on the nest and nursing (Brummelte and Galea,

2010a). Thus, early stress or exposure to glucocorticoids can impact the developmental outcome of the offspring directly and indirectly by reducing maternal care (Fleming et al., 1997; Liu et al., 1997; Nomura et al., 2002).

Direct administration of glucocorticoids to the dam, rather than stress exposure, will allow us to better understand the contribution of glucocorticoids on the effects of pre- or postnatal stress. Antenatal steroids are used in threatened preterm labor to support the lung surfactant maturation (Roberts and Dalziel, 2006), but it has been suggested that multiple dosages could have a negative influence on the development of the infant (Wapner et al., 2007). From animal studies, it is well known that administration of glucocorticoids directly to the offspring during early postnatal development can cause permanent morphological, physiological, and behavioral modifications (Edwards and Burnham, 2001; Machhor et al., 2004; Theogaraj et al., 2005). These modifications include a decrease in brain weight (DeKosky et al., 1982; Ferguson and Holson, 1999) , long-term and selective down-regulation of glucocorticoid receptors (Felszeghy et al., 1996), impaired adrenocortical response to stress (Erskine et al., 1979), disruptions in learning (DeKosky et al., 1982; Vicedomini et al., 1986), and delayed development (Golub, 1982; Pavlovska-Teglia et al., 1995). However, less is known on how maternal glucocorticoid levels affect the offspring, as the offspring may be partly buffered from maternal glucocorticoids during the first two weeks of life, a period known as the 'stress hypo-responsive period' (Sapolsky and Meaney, 1986).

We have shown previously that administration of high CORT to dams postpartum leads to reduced hippocampal cell proliferation in male pre-adolescent offspring and behavioral changes in adult male and female offspring (Angelucci et al., 1983; Brummelte et al., 2006). We further showed that CORT treatment to the dam during pregnancy resulted in increased serum CORT in the offspring shortly after birth (Brummelte et al., 2010). Interestingly, maternal

postpartum treatment with CORT increased whole brain levels of CORT on day 7 but no significant differences were seen in CORT levels in the prefrontal cortex, hypothalamus or hippocampus in 18 day old pups from CORT-treated dams compared to controls (Brummelte et al., 2010). Further we have found that treatment with high CORT postpartum, but not during gestation, results in a depressive phenotype in the dam (Brummelte and Galea, 2010a), suggesting that the effects of elevated maternal glucocorticoids on behavior and glucocorticoid levels in the offspring may also depend on the time of exposure during gestation or postpartum.

The present study was conducted to investigate the effects of two doses of CORT (10mg/kg; 40mg/kg) administered to dams during gestation, postpartum or both periods on behavioral and neuroendocrine parameters in male and female offspring. For this, dams received daily injections of either oil or CORT (low or high dose) from gestational day 10-20 and/or from postnatal day 2-24. Offspring were tested on the forced swim test (depressive-like behavior), the open field test (locomotor and anxiety-like behavior), the elevated plus maze (anxiety-like behavior), and the resistance to capture test (impulsivity/aggressiveness) during late adolescence or given restraint stress during adulthood. We hypothesized that CORT administered during the postpartum period would have a different effect on the offspring than CORT administered to dams during pregnancy. Further, we expected that high maternal CORT would have a stronger effect than low maternal CORT on the offspring and that the prolonged exposure (pregnancy and postpartum) of CORT would have a more substantial effect on the offspring than during pregnancy or the postpartum alone. We also hypothesized that males and females will show different vulnerabilities for high maternal glucocorticoid levels depending on the time of exposure.

2. Materials and methods

*2.1. **Animals***

Forty-six female and twenty male Sprague-Dawley rats, approximately 3 month old, were obtained from the University of British Columbia Animal Care Facility (Vancouver, Canada) for breeding. Rats were housed initially in same sex pairs in opaque polyurethane bins (48 × 27 × 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.). The number of animals was based on previous work in our laboratory (Brummelte et al., 2006) to give us sufficient power for the analysis. 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. All efforts were made to minimize animal suffering and to reduce the number of animals used. For an overview of the experimental procedures see Figure 1.

*2.2. **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 elevates blood levels of CORT for a prolonged period of time (Johnson et al., 2006; Sapolsky, 1985), increases depressive-like behavior (Brummelte et al., 2006; Brummelte and Galea, 2010a; Gregus et al., 2005) and results in serum levels of CORT consistent with ether stress in postpartum females (Yeh, 1984). The low dose was chosen because it does not alter depressive-like behavior or baseline CORT levels in male or female rats after s.c. injections, but it has been shown to diminish their CORT-response to stress suggesting this dose mimics CORT levels of a

mild chronic stressor (Brummelte and Galea, 2010b; Johnson et al., 2006). However, it is important to note, that oral application of CORT hemisuccinate through drinking water equivalent to a dose of 5-7mg/kg for 20 days results in a depressive phenotype in rats and mice 2-4 weeks after the cessation of treatment (Gourley et al., 2008; Gourley and Taylor, 2009).

2.3. ***Procedures***

Detailed breeding procedures and dam treatment has been described previously (Brummelte and Galea, 2010a). Briefly, pregnant dams were either given no injection (Undisturbed) or daily subcutaneous (s.c.) injections (1ml/kg) of either oil, low CORT (10mg/kg) or high CORT (40mg/kg). Subcutaneous injections were performed daily around 10 a.m. from day 10-20 of pregnancy and day 2-24 of postpartum. Rats were randomly assigned to one of the following groups: Undisturbed (n=5); Oil (n=10): received sesame oil injections during pregnancy and postpartum; Preg10 (n=5): received low CORT during pregnancy and oil injections during postpartum; Post10 (n=5): received sesame oil during pregnancy and low CORT during the postpartum period, P+P 10 (n=5): received low CORT during pregnancy and the postpartum, Preg40 (n=5) received high CORT during pregnancy and oil during postpartum, Post40 (n=5): received oil during pregnancy and high CORT during the postpartum period and P+P 40 (n=5) received high CORT during pregnancy and the postpartum. For an overview of the groups see Supplementary Table S1. The day the litter was born was considered postnatal day (PND) 0. On PND1 all litters were weighed and male and female pups were counted before culling the litter to 5 males and 5 females per dam. 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 when the litter was culled, the dam kept an uneven number of male and female pups (2 cases). The litter was weighed every 4 days starting

PND9 until PND20. Afterwards (including weaning) males and females were weighed separately. On postpartum days 2 to 8 maternal behavior was assessed as previously described in detail (Brummelte and Galea, 2010a). Time spent licking, nursing, or off pups was scored twice a day for 10 min. Dams were moved to maternal bins (48 x 40 x 27) with their litters on postnatal day 9. Pups were weaned on day 23 and group housed with same sex siblings for one week before they were pair-housed with same-sex siblings in standard clear polyurethane bins (Fig.1).

2.4. ***Behavioral testing of the offspring***

Starting at a mean age of 42.35 ± 0.25 days 8 male and 8 female offspring from each group for a total of 128 rats were tested in a battery of behavioral tests including the forced swim test, open field test, elevated plus maze and resistance to capture test during late adolescence (~40-60d). No more than 2 males and 2 females were taken from each litter for the behavioral tests. Behavioral tests were conducted in the same order for all animals (see Fig. 1) and were completed after ~20days with at least 2 days break between each test for each animal. In early adulthood (age ~70d) one male/female (n=5/group) pup from each litter (which had not been used for behavioral testing) underwent 1 hour of restraint stress and baseline and stress response levels of CORT were measured.

2.4.1. Forced swim test

The forced swim test (FST) was used to assess depressive-like behavior in the offspring and was used as previously described (Galea et al., 2001; Hansen et al., 1997). 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 ± 0.5°C. Session 1 of the FST lasted 15 min and was followed 24 hours

later by a 2nd session lasting 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.

2.4.2. Open Field Test

On a separate day following the 2nd FST session and in order to assess general locomotor activity the open field test (OFT) was used as previously described (Galea et al., 2001). Briefly, the apparatus, a 120×120 cm^2 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. Rats were placed in the field facing a corner and locomotor activity was video recorded for a 10 min period. An observer blind to conditions scored the number of rears and the number of fecal boli from each animal. The videos were analyzed with Anymaze (Stoelting Co., Wood Dale, USA), software for automatic behavioral scoring to assess the total distance travelled by the animal and time spent in each zone.

2.4.3. Elevated Plus Maze

On a day following OFT but still during adolescence (~50d), pups were tested in the Elevated Plus Maze (EPM) as described previously (Brummelte et al., 2006; Pellow et al., 1985). Briefly, rats were placed in the center of a plus-shaped apparatus with two open and two enclosed arms for 5 minutes. The number of entries into open or closed arms was counted as well as the

time spent in a closed arm, open arm or the center. The amount of time spent in a closed arm vs. time spent in open arm or center was used as an index of anxiety.

2.4.4. Resistance to capture

Resistance to capture scale from Kalynchuk et al. (1997) was adjusted and performed as previously described (Brummelte et al., 2006). Briefly, a few days after the OFT animals were placed into an unfamiliar small open field (50cm x 50 cm) and were allowed to explore it freely for 2 min. Then an experimenter approached them with an unfamiliar large leather glove and tried to pick them out of the field. Behaviors were scored according to a 7-point scale (0= easy to pick up/ 7= manage to escape from grip, Supplementary Table S2) at three different time points: approach of the glove, touch and lifting. In addition, the time to capture was recorded.

2.4.5. Restraint stress

To assess the young adult offspring's CORT response to stress, separate rats were examined that had not undergone behavioral tests. Five males and 5 females from each group were subjected to 1 hour of restraint stress at age 66-73 days. A baseline blood sample was collected prior to placing the rat into the tube (t0). All samples were collected within 3 min after touching their home cage to avoid a rise in CORT levels due to the stress of tail bleeding. Further samples were taken at 30min (t30) into the stress, at the end of the stressor (t60), and 1 hour after (t120) their return to their home cage. All blood samples were collected from the tail vein and in under 3 minutes.

2.5. ***Corticosterone assay***

Blood samples were stored overnight at 4°C to allow blood to clot completely, centrifuged at 10,000 × g for 10 min, serum collected and stored at -20°C until further processing. Total CORT (bound and free) was measured using the ImmuChem Double Antibody ^{125}I radioimmunoassay Kit (MP Biomedicals, Orangeburg, NY, USA). The antiserum cross-reacts 100 % with CORT, 0.34 % with deoxycorticosterone, 0.10 % with testosterone, 0.10 % with cortisol, but does not cross-react with progesterone and estrogens (< 0.01%). The intra-assay and inter assay coefficients of variation were less than 7.1% and 6.5% respectively. All reagents were halved and samples run in duplicates.

2.6. ***Data analyses***

The average weight per pup was calculated by dividing the litter weight after culling by the number of pups per litter (n=10). Changes in body weight during the postpartum was analyzed with a repeated-measures ANOVA with group (Control, Oil, Preg10, Post 10, P+P10, Preg40, Post40, P+P40) as the between-subjects factor and age (PND1, PND9, PND13, PND17, PND20) as the within-subject factors. Analysis of FST, OFT, EPM and resistance to capture was performed using repeated-measure ANOVAs with FST behavior (struggling, swimming, immobile), OFT number of crossings and distance travelled (peripheral, center), EPM arm entries (open, closed) and time spent in each zone (open, closed, center) as the within-subject factors and group and sex (male, female) as the between-subject factors. Further, number of rears (EPM and OFT), time spent in center (OFT), central/total distance ratio (OFT), time in closed/time in open arm or center (EPM) and number of fecal boli (OFT and resistance to capture), capture behavior (cumulative score and time to capture in resistance to capture) were each analyzed separately using a one-way ANOVA. Serum CORT levels during restraint stress were analyzed using a repeated-measures ANOVA with time (t0, t30, t60, t120) as the within subject factor and group

as the between-subjects factor. Pearson product-moment correlations were conducted between offspring behaviors and maternal care behavior (% of time spent off the nest). Tests of homogeneity of variance (using the Brown-Forsythe's test), normality (using the Kolmogorov-Smirnov test) and in the cases where more than two dependent variables were analyzed in a repeated-measures ANOVA, tests of sphericity were conducted. Violations to these assumptions are noted in the text however it should be noted that the ANOVA is quite robust to violations of normality when using a fixed-effects ANOVA (Glass and Hopkins, 1995), as in the present case. When violations were noted in sphericity we used the Huynh-Feldt adjustment, while violations of homogeneity of variance used the Welch's test.

All post-hoc tests utilized LSD. All figures show data separately for males and females regardless of significant main or interaction effects of sex.

3. Results

3.1. ***Body weight - Offspring from dams exposed to high CORT showed attenuation of weight gain throughout development. At adulthood, offspring from dams exposed to low CORT weighed less than controls.***

Litter characteristics from the current offspring have been published previously (Brummelte and Galea, 2010a) and are shown in Supplementary Table S3. Detailed results of the post-hoc analysis for body weight development during the postpartum period are shown in Table 1. There was no significant difference in body weight between the groups on postnatal day 1 (all p's>0.46), but starting on PND9 or PND 13 all high dose groups and Post10 showed attenuated weight gain (all p's<0.02). Results of the repeated-measures ANOVA were as follows: main effects for group ($F(7, 38)=7.26$, $p<0.001$) and age ($F(4, 152)=2522.3$, $p<0.001$) and a significant

interaction of group and age ($F(28, 152)=3.56$, $p<0.001$). There was a violation of sphericity ($p<.001$), however the Huynh-Feldt adjusted p value for the age by group interaction was $p<0.0001$). There were no other violations in the assumptions of the ANOVA.

At weaning (postnatal day 23) Post10, Preg40, Post40 and P+P40 pups weighed less than undisturbed or oil controls (all p's <0.008; Figure 2A). The ANOVA revealed significant main effects of group ($F(7, 76)=16.07$, $p<0.0001$) and sex ($F(1, 76)=6.67$, $p=0.01$) with males being significantly heavier than females, but no significant interaction effect ($p=0.80$). There were no violations of the assumptions of the ANOVA.

Animals were weighed again in early adulthood (day 72 ± 0.25) and body weight was analyzed with an ANCOVA correcting for age. All low CORT groups weighed significantly less compared to oil rats (all p's <0.01), but the high CORT groups were not different from oil controls (all p's >0.66) (Figure 2B). There was no significant interaction effect ($F(7,75)=1.26$, $p=0.28$), but a main effect of sex ($F(1,75)=973.6$, $p<0.001$), with males weighing more than females, and a significant group effect ($F(7,75)=3.22$, $p= 0.005$). There were no violations of the assumptions of the ANOVA.

3.2. ***Forced Swim Test (FST) – Offspring from dams exposed to high CORT during gestation, but not postpartum, exhibited depressive-like behavior in the FST.***

Due to the heterogeneity of variance the data were transformed with the arcsine transformation for the analysis. The Preg40 group showed an increase in immobility and decrease in swimming compared to undisturbed (p's=0.01) or oil controls (p's<0.002; Figure 3). The repeated measure ANOVA revealed a significant interaction effect of forced swim test behavior and group ($F(14, 254)=2.16$, $p=0.009$) and a main effect of behavior ($F(2, 254)=204.1$,

p<0.001), but no significant main effect of sex or other interaction effects (all p's>0.13). Because there was a violation of sphericity and the Huynh-Feldt adjusted p value for the behavior by group interaction was p<0.02. There were no other violations in the assumptions of the ANOVA.

3.3. ***Open Field Test (OFT): Offspring from dams exposed to CORT during gestation and the postpartum travelled greater distances in the periphery of the open field compared to controls.***

For peripheral distance, oil females travelled greater distances than Post10 females (p=0.004) and undisturbed controls (p=0.01). P+P10 and P+P40 females travelled significantly more in the periphery than undisturbed controls (p<0.001; p=0.005, respectively) and oil (p=0.009, P+P10 females only). For males, P+P10 travelled significantly more in the periphery compared to male oil controls (p=0.05), but Preg40 crossed significantly less than oil controls (p=0.02) or undisturbed controls (p=0.01; Figure 4). There were no significant differences between the groups for the distance travelled in the center of the open field (Table 2). Repeated-measures ANOVA for distance travelled showed significant main effects for group (F(7,121)= 2.28, p=0.03), sex (F(1,121)=28.04, p<0.001) and area (F(1, 121)=5229.2, p<0.001), significant two-way interaction effects for group and area (F(7,121)=2.17, p=0.04), sex and area (F(1,121)=12.25, p<0.001) and a significant three-way interaction for group, sex and area (F(7,121)=2.08, p=0.05). Further, ANOVAs showed that males showed significantly more fecal boli (p<0.001), less rearing (p=0.01), less time in the center (p<0.001), lower center/total distance ratio (p<0.001) and longer latency to enter the center (p=0.02) in the open field test (Table 2). There were no violations to assumptions of the ANOVA.

3.4. ***Elevated Plus Maze (EPM): Male, but not female, offspring from dams exposed to high CORT during gestation and the postpartum or just the postpartum, exhibited anxiety-like behavior in the EPM.***

Preg10 and Post10 rats made overall fewer entries into the open or closed arms than oil rats (p's<0.02) (Table 3). For the time spent in each arm, males from dams that received high CORT postpartum spent more time in closed arms compared to oil controls (Post40: p=0.03; P+P40=0.004). For females, only the Post40 group spent significantly more time in the closed arms (p=0.04), and P+P10 spent more time in the center compared to oil controls (p=0.02). The time in a closed arm vs. time in open arm or center was used as an index of anxiety with a higher ratio indicating more anxious behavior. Post40 and P+P40 males had significantly higher closed/open ratios compared to undisturbed or oil controls (p's <0.02), while no such effect was seen in females (p's >0.39, Figure 5). Repeated-measures ANOVA for the entries into the open or closed arm showed a significant main effect of group ($F(7, 128)=2.66$, $p=0.01$), sex ($F(1, 128)=14.1$, $p<0.001$) and arm ($F(1,128)=1087.2$, $p<0.001$), with more closed than open arm entries and females making more entries than males. For the time spent in each zone (closed arm, open arm or center) repeated measures ANOVA showed a significant interaction effect of group and zones ($F(14, 248)=3.41$, $p<0.001$) sex and zones ($F(2, 248)=4.48$, $p=0.01$) and a significant three-way interaction ($F(14, 248)=1.78$, $p=0.04$). Closed/open ratios showed an interaction effect of group and sex ($F(7, 124)=2.51$, $p=0.02$) main effect of sex ($F(1, 124)=4.67$, $p=0.03$) and group ($F(7, 124)=3.40$, $p=0.002$). There were no violations to homogeneity of variance but the open arm entries and time spent in open arms deviated from normality ($p<.05$).

3.5. ***Resistance to capture - Offspring from dams exposed to high CORT had higher capture scores compared to controls.***

Compared to oil controls, P+P10 animals were caught faster ($p=0.006$), while Preg40 animals took longer to be caught ($p=0.02$), and all high CORT groups (i.e. Preg40, Post40, and P+P40) had higher cumulative capture scores compared to oil and undisturbed controls (all p's <0.03; Figure 6). There was a significant group effect for the time to capture the animal ($F(7, 126)=3.87$, $p<0.001$) and the cumulative capture score ($F(7, 126)=3.52$, $p=0.002$) but no sex or interaction effects (p's >0.05). There was heterogeneity of variance for total score ($p<0.015$) but not for time to capture. However, all of the effects noted above were still significant using the Welch's test.

3.6. ***Restraint stress - Offspring from dams exposed to CORT during gestation had lower basal CORT while offspring from dams exposed to low CORT postpartum had higher basal CORT. Offspring from dams exposed to CORT during gestation or low CORT during gestation and the postpartum had higher stress levels of CORT.***

Preg10 and Preg40 animals had lower baseline (t0) levels compared to undisturbed controls (both p's=0.02), but not compared to oil controls (p's>0.17) while Post10 and P+P10 rats had higher baseline levels (t0) compared to oil controls (p's<0.001). Preg10, Preg40 and P+P10 groups had higher peak levels at the end of restraint stress (t60) compared to oil controls (all p's<0.02). P+P10 had also significantly higher recovery (t120, one hour after end of restraint stress) levels compared to oil controls (p's <0.004), while all high CORT groups had lower recovery levels (t120) compared to undisturbed controls (p's<0.05). There was no difference between undisturbed and oil controls for any time point (all p's>0.12). For a full display of all significant differences see Figure 7. Repeated measures ANOVA with time (t0, t30,t60 and t120) as within-subject factors revealed significant main effects of group ($F(7,71)=4.93$, $p<0.001$), sex ($F(1,71)=163.25$, $p<0.001$) and time ($F(3,213)=423.58$, $p<0.001$) and significant interaction

effects between group and sex ($F(7,71)=2.17$, $p=0.05$), sex and time ($F(3,213)=16.11$, $p<0.001$), and time and group ($F(7,213)=1.61$, $p=0.05$), but not between all three factors ($p=0.65$). Mauchley's test for sphericity was significant, however the Huynh-Feldt adjustment indicated significant effects for all the within-subjects factors (adjusted p's all <0.014). There were no violations of homogeneity of variance, but normality was violated for t120 in males only.

Table 4 gives an overview of the directions of effects of selected tests and behaviors.

3.7 ***Correlation of maternal care with behavioral outcome***

Detailed results of maternal behavior have been reported previously (Brummelte and Galea 2010a). Briefly, the P+P10, Preg40, Post40 and P+P40 groups spent more time off the nest (all p's< .01) and less time nursing (all p's< .05) than oil controls, while there was no significant difference in amount of time spent licking for any of the groups (all p's>.71). The peripheral distance crossed in the OFT was not associated with maternal care overall ($p=0.86$), but looking at the correlations by group, the peripheral distance was negatively associated with the dams' time spent off the nest for the Preg10 group ($r= -0.58$, $p=0.017$) and positively associated for the Post10 group ($r=0.58$, $p=0.019$). Total capture score in the resistance to capture test was slightly but significantly correlated with maternal time spent off the nest ($r= 0.19$, $p=0.02$), however there were no significant correlations for maternal behavior and resistance to capture behavior of the offspring by group (all p's>0.18). There was a significant correlation for the recovery CORT levels (t120: $r=-0.20$, $p=0.05$) after restraint stress and maternal time off the nest. Analysing this correlation for each group separately showed that for the oil group, offspring from mother spending more time off the nest had lower CORT levels at t120 ($r= -0.61$, $p=0.006$).There were no other significant correlations.

4. Discussion

The results from the present study demonstrate that exposure to maternal exogenous CORT during gestation or the postpartum leads to dose-dependent and time of administration-dependent changes in the male and female offspring. To our knowledge this is the first report on consequences of elevated maternal CORT levels throughout pregnancy and the postpartum for the male and female offspring. High levels of maternal CORT during pregnancy, but not the postpartum, lead to more depressive-like behavior in the offspring, while high maternal CORT during the postpartum lead to more anxiety-like behavior in the male, but not female, offspring compared to controls. Further, all high CORT groups showed more impulsive-like behavior as measured by the resistance to capture test. Both male and female rats exposed to CORT during pregnancy had lower adult baseline levels and higher stress corticosterone levels, while rats exposed to low CORT during the postpartum had higher baseline CORT levels. Weight gain during the postpartum period was significantly attenuated in offspring exposed to maternal CORT pre- or post-natally. Interestingly, in adulthood only the low CORT groups had reduced weight compared to controls. Overall, our results demonstrate that the impact of maternal CORT on the offspring depend on the dose and time of exposure, with varying effects of antenatal, postnatal or prolonged exposure during gestation and the postpartum period.

4.1. Offspring from dams exposed to high CORT showed attenuation of weight gain throughout development; however as adults all the offspring from dams exposed to low CORT weighed less than controls.

In line with other studies, our results showed that early CORT exposure can affect the weight gain of the offspring (Brummelte et al., 2006; Franko et al., 2010). Offspring from dams exposed

to high CORT either during gestation or postpartum showed attenuation of weight gain throughout development. However, as adults all the offspring from dams exposed to low CORT, either during gestation or postpartum, weighed less than controls. These findings suggest that different levels of CORT can differentially affect or re-program the metabolic system (Franko et al., 2010), which is in line with previous studies showing varying results of chronic CORT on weight depending on the dose, timing, route of administration and type of glucocorticoid used (Brummelte and Galea, 2010a; Catalani et al., 1993; Karatsoreos et al., 2010). Our findings are further consistent with studies examining the effect of maternal stress on offspring body weight (Baker et al., 2009; Mueller and Bale, 2006). For example, maternal stress during late pregnancy temporarily changed the offspring's body weight, while stress experienced earlier in pregnancy resulted in long-term increase in body weight in male offspring of stress-sensitive dams (Mueller and Bale, 2006).

4.2. High levels of maternal CORT during pregnancy leads to more depressive-like behavior in the offspring

Our finding of more depressive-like behavior in the Preg40 group is in concert with many studies showing more depressive-like behavior after prenatal stress in rodents (Abe et al., 2007; Alonso et al., 2000; Secoli and Teixeira, 1998). Further, one prospective longitudinal study in humans found that adolescents who had been exposed to antenatal depression had a 4.7 times higher risk for being diagnosed with a depressive disorder compared to non-exposed adolescents (Pawlby et al., 2009). However, direct glucocorticoid administration to pregnant dams does not always produce behavioral despair in the offspring (e.g. Hauser et al., 2009) and we did not observe increased-depressive-like behavior in our Preg10 group which were also exposed to elevated CORT *in utero*. Intriguingly, Wilcoxon and Redei (2007) demonstrated that both

adrenalectomy (ADX) of the dam and ADX plus high CORT replacement lead to increased immobility in the forced swim test. Therefore, the mechanism underlying the effect of maternal glucocorticoids on depressive-like behavior in the offspring is complex and it is conceivable that there is a curvilinear relationship between maternal CORT levels and behavioral outcome. Along with increased levels of immobility in the FST, the Preg40 group also had decreased time spent swimming in the FST. Swimming behavior in the FST is mediated through serotonin levels (Detke et al., 1995), indicating that serotonin levels may be dysregulated in offspring exposed to high maternal CORT. Chronically elevated CORT levels are believed to impair serotonergic neurotransmission (Meijer and de Kloet, 1998), thus it is tempting to suggest that the decrease in swimming in the Preg40 group may be due to CORT-induced alterations in serotonergic transmissions. The fact that there was no effect of postnatal CORT exposure on immobility in the forced swim test in male or female offspring is in line with our previous study (Brummelte et al., 2006). It should be noted that while the FST is well established as a behavioral indicator of antidepressant efficacy (Cryan et al., 2005; Porsolt et al., 1978) where decreased immobility is associated with greater efficacy for antidepressant properties, many researchers have used the corollary to suggest that increased time spent immobile in the FST is indicative of depressive-like behaviors (Brummelte et al., 2006; Galea et al., 2001; Kalynchuk et al., 2004; LaPlant et al., 2009; Stoffel and Craft, 2004). However the use of FST as an index of depressive-like behavior is not without controversy as it is not clear exactly what immobility in the FST is modeling (for review see: Cryan et al., 2005).

4.3. High levels of postpartum CORT increase anxiety-like behavior in males

Post40 and P+P40 males spent more time in the closed arm of the Elevated Plus Maze and had higher closed/open arm ratio both of which are indicative of more anxiety-like behavior.

This finding is not due to these animals showing less locomotor activity as they did not differ from controls regarding the distance travelled in the Open Field Test. This effect is in concert with the literature showing higher anxiety after early stress exposure (Lukkes et al., 2009; Wei et al., 2010) but our study is the first to demonstrate that high CORT postpartum (or during gestation and postpartum) leads to more anxiety-like behavior in the male adolescent offspring.

The higher resistance to capture scores in the high CORT offspring compared to oil controls could also indicate more anxious and/or impulsive behavior in these animals (Brummelte et al., 2006; Kalynchuk et al., 1997). Stone and colleagues (1988) demonstrated increased escape behavior after chronic administration of CORT to adult rats and reduced escape behavior after adrenalectomy, suggesting that CORT may play a role in maintaining flight responses during chronic stress. Taken together our results show that early CORT exposure has a complex impact on anxiety-related behaviors, which may be due to different developmental maturation patterns of associated brain structures, such as the amygdala or the prefrontal cortex. These brain areas may be more vulnerable to excessive glucocorticoids at different time points during development.

4.4. *Adult offspring exposed to low and high CORT in utero had lower basal but higher stress levels of CORT*

Adult offspring given one hour of restraint stress revealed changes in basal and stress levels of CORT that depended on time of maternal exposure to CORT (gestation or postpartum) and the dose of CORT. Exposure of the dam to low CORT postpartum upregulated basal serum CORT levels in the offspring, while exposure of the dam to CORT antepartum decreased basal serum CORT in the offspring. Exposure of the dam to high CORT downregulated the CORT

response during recovery in the offspring, resulting in CORT levels below those of the undisturbed or oil controls, which was more particularly evident in the females. However exposure of the dam to low or high CORT antepartum resulted in an upregulated CORT response during the one hour of stress. Consistent with previous literature (Matthews et al., 2004; Meaney et al., 2007) this suggests that the function of the HPA axis is programmed differently during *in utero* versus *ex utero* exposure to elevated maternal glucocorticoids. Prenatal exposure to high levels of glucocorticoids might prepare the offspring for a challenging environment by reprogramming the HPA axis to being hyposensitive to mild stressors. Exposure to high levels of maternal glucocorticoids during the postpartum on the other hand may cause different "programming" as the HPA axis is further matured at this stage and may be less plastic. In line with this, chronic low CORT exposure during adolescence attenuates the physiological stress response in adulthood (Xu et al., 2010). In addition maternal contact can inhibit stress responsiveness and that maternal stimuli play a special role in the modulation of pituitary-adrenal activity during development (Stanton and Levine, 1990), thus it is possible that the reduced maternal care/contact we observed in the dams (Brummelte and Galea, 2010a) also contributed to programming the HPA axis of the offspring during the postpartum period.

4.5. Sex differences in the effects of elevated maternal CORT on the offspring

Our results show that the effects of CORT treatment in dams were not always consistent between the sexes. This is consistent with several studies that have reported sex differences after an early glucocorticoid treatment (Golub, 1982; Scheff et al., 1988; Vicedomini et al., 1986). Furthermore, Kalynchuk and her colleagues (2004) found that adult male rats treated with high CORT for three weeks exhibited stronger physiological effects than treated females. Males

generally appear to be more vulnerable to the effects of acute and chronic stress, depending on the nature of the stressor and the age of the animal (Falconer and Galea, 2003; Galea et al., 1997). Possible reasons for this are several sex differences in the endocrine system and HPA axis activity. It is well known that females show higher basal and stress ACTH, CORT and corticosteroid binding globulin levels than males (Duncko et al., 2001; Gala and Westphal, 1965). Further, females appear to be more resistant to the damaging effects of stress at certain periods in their life (Bowman, 2005) and show a dampened response of their HPA axis during reproduction, which may be an evolutionary mechanism to protect the offspring (Brunton et al., 2008; Young and Cook, 2004). A sex-dependent effect of stress would be in line with previous human studies showing a more pronounced impact of postnatal or antenatal depression on behavioral and cognitive development in boys compared to girls (Gerardin et al., 2011; Murray and Cooper, 1997).

4.6. Early life maternal conditions change adult phenotype: lessons from the CORT-induced model of stress and/or depression

We have previously shown that chronic exposure to CORT postpartum leads to increased depressive-like behavior, reduced maternal care, reduced hippocampal neurogenesis and reduced weight gain in dams and may thus serve as an animal model of postpartum depression with high face validity (Brummelte et al., 2006; Brummelte and Galea, 2010a). The offspring from this model show sex-dependent significant changes in certain behaviors and alterations in their stress response which indicates adaptation of the pups to the early adverse conditions. In line with our results, Petropoulos and colleagues (2010) have recently demonstrated that synthetic glucocorticoid administration during pregnancy can alter levels of blood-brain barrier proteins in a complex dose- time- and sex-specific manner, indicating that all three factors may play a

crucial role in determining the impact of glucocorticoids on brain maturation. Glucocorticoids are indispensable for early development and essential for many peripheral and neuronal maturation processes (McEwen, 1987). Importantly, even if a single course of antenatal steroid is considered safe and does not alter HPA axis function (Dalziel et al., 2005; Gover et al., 2011), repeated doses of synthetic antenatal steroids have been associated with restricted growth and altered cardiovascular, HPA and metabolic function (for review see: Sloboda et al., 2005). An increase in endogenous glucocorticoids, as seen after prenatal stress, can lead to earlier earflap and eye opening and faster righting in rat pups (Secoli and Teixeira, 1998), which suggests that increased glucocorticoids levels accelerate maturation of the fetus beyond lung maturation. An accelerated development could represent an evolutionary mechanism to adapt to a challenging environment. Interestingly, in our study the prolonged exposure to CORT during pregnancy and the postpartum did not result in accumulated effects in the offspring. This may be due to the possibility that the exposure during pregnancy already prepared them for a more adverse postnatal environment. In line with this, King and colleagues (2004) showed that maternal malnutrition during pregnancy prevented the effects of acute postnatal stress on neurogenesis in the dentate gyrus. Overall, the offspring from our study seemed to be less affected compared to other studies using synthetic glucocorticoids or direct exposure of the offspring (e.g. DeKosky et al., 1982; Flagel et al., 2002; Hauser et al., 2009), which might be due the lower potency and better protection from natural glucocorticoid compared to synthetic ones which can for instance easily cross the placental barrier (Seckl et al., 2000). Further, we cannot exclude the possibility that CORT-induced changes in maternal care may have contributed to or caused the observed behavioral and neuroendocrine changes in the offspring. We found differences in maternal behavior between the groups (Brummelte and Galea, 2010a) and correlations between the time dams spent off the nest and resistance to capture behavior and HPA axis function of the

offspring. Unfortunately the present design prevents us from teasing apart the relative contributions of maternal CORT to directly or indirectly influence offspring behavior, but future studies could investigate this. Considering that high maternal CORT results in high CORT in the offspring (Brummelte et al., 2010) it is likely that the observed effects in the offspring are dependent on the multifaceted combination of glucocorticoid exposure, maternal behavior and environment.

5. Conclusions

Taken together, findings from our study show that maternal CORT impacts the behavioral and endocrine outcome of the offspring which is dependent on the dose, time of exposure to maternal CORT and on the sex of the offspring. Male, but not female, offspring of dams who were exposed to high CORT postpartum exhibited greater anxiety-like behavior, while offspring of dams given high CORT during gestation exhibited greater depressive-like behavior. This indicates that there are different critical windows for early exposure to glucocorticoids to influence anxiety or depressive-like behavior in the adult offspring. The timing of exposure to maternal CORT was also important in the manifestation of basal and stress CORT levels in adulthood as offspring from dams exposed to CORT during gestation had lower basal, but higher stress levels of CORT, while offspring from dams exposed to low, but not high, CORT postpartum had higher levels of basal CORT. Intriguingly the Preg40 group which showed more depressive-like behavior also had altered HPA axis activation in response to restraint stress, while the more ‘anxious’ Post40 and P+P40 males showed no such changes. It is important to better understand how early adverse experiences reprogram the offspring to prepare it for its subsequent environment as this will help to determine risk factors for neuropsychiatric diseases such as depression and anxiety disorders and health outcomes in future generations.

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Conflict of interest

The authors have nothing to declare.

Reference List

Abe H, Hidaka N, Kawagoe C, Odagiri K, Watanabe Y, Ikeda T, Ishizuka Y, Hashiguchi H, Takeda R, Nishimori T, Ishida Y (2007) Prenatal psychological stress causes higher emotionality, depression-like behavior, and elevated activity in the hypothalamo-pituitary-adrenal axis. Neurosci Res 59:145-151.

Alonso SJ, Damas C, Navarro E (2000) Behavioral despair in mice after prenatal stress. J Physiol Biochem 56:77-82.

Angelucci L, Patacchioli FR, Chierichetti C, Laureti S (1983) Perinatal mother-offspring pituitary-adrenal interrelationship in rats: corticosterone in milk may affect adult life. Endocrinol Exp 17:191-205.

Baker S, Rees S, Chebli M, Lemarec N, Godbout R, Huta V, Bielajew C (2009) Effects of gestational stress: 2. Evaluation of male and female adult offspring. Brain Res 1302:194-204.

Bosch OJ, Musch W, Bredewold R, Slattery DA, Neumann ID (2007) Prenatal stress increases HPA axis activity and impairs maternal care in lactating female offspring: implications for postpartum mood disorder. Psychoneuroendocrinology 32:267-278.

Bowman RE (2005) Stress-induced changes in spatial memory are sexually differentiated and vary across the lifespan. J Neuroendocrinol 17:526-535.

Brummelte S, Galea LA (2010a) Chronic corticosterone during pregnancy and postpartum affects maternal care, cell proliferation and depressive-like behavior in the dam. Horm Behav 58:769-779.

Brummelte S, Galea LA (2010b) Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. Neuroscience 168:680-690.

Brummelte S, Pawluski JL, Galea LA (2006) High post-partum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation and behavior of offspring: A model of post-partum stress and possible depression. Horm Behav 50:370-382.

Brummelte S, Schmidt KL, Taves MD, Soma KK, Galea LA (2010) Elevated corticosterone levels in stomach milk, serum, and brain of male and female offspring after maternal corticosterone treatment in the rat. Dev Neurobiol 70:714-725.

Brunton PJ, Russell JA, Douglas AJ (2008) Adaptive responses of the maternal hypothalamic-pituitary-adrenal axis during pregnancy and lactation. J Neuroendocrinol 20:764-776.

Catalani A, Marinelli M, Scaccianoce S, Nicolai R, Muscolo LA, Porcu A, Koranyi L, Piazza PV, Angelucci L (1993) Progeny of mothers drinking corticosterone during lactation has lower stress-induced corticosterone secretion and better cognitive performance. Brain Res 624:209-215.

Cryan JF, Valentino RJ, Lucki I (2005) Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev 29:547-569.

Dalziel SR, Walker NK, Parag V, Mantell C, Rea HH, Rodgers A, Harding JE (2005) Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. Lancet 365:1856-1862.

Darnaudery M, Maccari S (2008) Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Res Rev 57:571-585.

DeKosky ST, Nonneman AJ, Scheff SW (1982) Morphologic and behavioral effects of perinatal glucocorticoid administration. Physiol Behav 29:895-900.

Detke MJ, Rickels M, Lucki I (1995) Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl) 121:66-72.

Duncko R, Kiss A, Skultetyova I, Rusnak M, Jezova D (2001) Corticotropin-releasing hormone mRNA levels in response to chronic mild stress rise in male but not in female rats while tyrosine hydroxylase mRNA levels decrease in both sexes. Psychoneuroendocrinology 26:77-89.

Edwards HE, Burnham WM (2001) The impact of corticosteroids on the developing animal. Pediatr Res 50:433-440.

Erskine MS, Geller E, Yuwiler A (1979) Effects of neonatal hydrocortisone treatment of pituitary and adrenocortical response to stress in young rats. Neuroendocrinology 29:191-199.

Falconer EM, Galea LA (2003) Sex differences in cell proliferation, cell death and defensive behavior following acute predator odor stress in adult rats. Brain Res 975:22-36.

Felszeghy K, Gaspar E, Nyakas C (1996) Long-term selective down-regulation of brain glucocorticoid receptors after neonatal dexamethasone treatment in rats. J Neuroendocrinol 8:493-499.

Ferguson SA, Holson RR (1999) Neonatal dexamethasone on day 7 causes mild hyperactivity and cerebellar stunting. Neurotoxicol Teratol 21:71-76.

Flagel SB, Vazquez DM, Watson SJ, Jr., Neal CR, Jr. (2002) Effects of tapering neonatal dexamethasone on rat growth, neurodevelopment, and stress response. Am J Physiol Regul Integr Comp Physiol 282:R55-R63.

Fleming AS, Ruble D, Krieger H, Wong PY (1997) Hormonal and experiential correlates of maternal responsiveness during pregnancy and the puerperium in human mothers. Horm Behav 31:145-158.

Franklin TB, Russig H, Weiss IC, Graff J, Linder N, Michalon A, Vizi S, Mansuy IM (2010) Epigenetic transmission of the impact of early stress across generations. Biol Psychiatry 68:408-415.

Franko KL, Forhead AJ, Fowden AL (2010) Differential effects of prenatal stress and glucocorticoid administration on postnatal growth and glucose metabolism in rats. J Endocrinol 204:319-329.

Gala RR, Westphal U (1965) Corticosteroid-binding globulin in the rat: Possible role on the initiation of lactation. Endocrinology 76:1079-1088.

Galea LA, McEwen BS, Tanapat P, Deak T, Spencer RL, Dhabhar FS (1997) Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. Neuroscience 81:689-697.

Galea LA, Wide JK, Barr AM (2001) Estradiol alleviates depressive-like symptoms in a novel animal model of post-partum depression. Behav Brain Res 122:1-9.

Gerardin P, Wendland J, Bodeau N, Galin A, Bialobos S, Tordjman S, Mazet P, Darbois Y, Nizard J, Dommergues M, Cohen D (2011) Depression during pregnancy: is the developmental impact earlier in boys? a prospective case-control study. J Clin Psychiatry 72:378-387.

Glass GV, Hopkins KD (1995) Statistical Methods in Education and Psychology. Allyn & Bacon.

Golub MS (1982) Maze exploration in juvenile rats treated with corticosteroids during development. Pharmacol Biochem Behav 17:473-479.

Gourley SL, Kiraly DD, Howell JL, Olausson P, Taylor JR (2008) Acute hippocampal brain-derived neurotrophic factor restores motivational and forced swim performance after corticosterone. Biol Psychiatry 64:884-890.

Gourley SL, Taylor JR (2009) Recapitulation and reversal of a persistent depression-like syndrome in rodents. Curr Protoc Neurosci Chapter 9:Unit.

Gover A, Brant R, Weinberg J, Whitfield MF, Brummelte S, Synnes AR, Miller SP, Grunau RE (2011) A single course of antenatal steroids does not alter cortisol on preterm infants. (unpublished results).

Gregus A, Wintink AJ, Davis AC, Kalynchuk LE (2005) Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. Behav Brain Res 156:105-114.

Gross CM, Flubacher A, Tinnes S, Heyer A, Scheller M, Herpfer I, Berger M, Frotscher M, Lieb K, Haas CA (2010) Early life stress stimulates hippocampal reelin gene expression in a sex-specific manner: Evidence for corticosterone-mediated action. Hippocampus (Epub ahead of print).

Hansen HH, Sanchez C, Meier E (1997) Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immobility in adult rats: a putative animal model of depression? J Pharmacol Exp Ther 283:1333-1341.

Hauser J, Feldon J, Pryce CR (2009) Direct and dam-mediated effects of prenatal dexamethasone on emotionality, cognition and HPA axis in adult Wistar rats. Horm Behav 56:364-375.

Johnson SA, Fournier NM, Kalynchuk LE (2006) Effect of different doses of corticosterone on depression-like behavior and HPA axis responses to a novel stressor. Behav Brain Res 168:280-288.

Kalynchuk LE, Gregus A, Boudreau D, Perrot-Sinal TS (2004) Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. Behav Neurosci 118:1365-1377.

Kalynchuk LE, Pinel JP, Treit D, Kippin TE (1997) Changes in emotional behavior produced by long-term amygdala kindling in rats. Biol Psychiatry 41:438-451.

Karatsoreos IN, Bhagat SM, Bowles NP, Weil ZM, Pfaff DW, McEwen BS (2010) Endocrine and physiological changes in response to chronic corticosterone: a potential model of the metabolic syndrome in mouse. Endocrinology 151:2117-2127.

King RS, DeBassio WA, Kemper TL, Rosene DL, Tonkiss J, Galler JR, Blatt GJ (2004) Effects of prenatal protein malnutrition and acute postnatal stress on granule cell genesis in the fascia dentata of neonatal and juvenile rats. Brain Res Dev Brain Res 150:9-15.

LaPlant Q, Chakravarty S, Vialou V, Mukherjee S, Koo JW, Kalahasti G, Bradbury KR, Taylor SV, Maze I, Kumar A, Graham A, Birnbaum SG, Krishnan V, Truong HT, Neve RL, Nestler EJ, Russo SJ (2009) Role of nuclear factor kappaB in ovarian hormone-mediated stress hypersensitivity in female mice. Biol Psychiatry 65:874-880.

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277:1659-1662.

Lukkes JL, Watt MJ, Lowry CA, Forster GL (2009) Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. Front Behav Neurosci 3:18.

Machhor N, Balaji T, Raju TN (2004) Postnatal dexamethasone and long term learning and memory functions in developing rats: Effect of postnatal age and gender. Life Sci 74:1925-1935.

Matthews SG, Owen D, Kalabis G, Banjanin S, Setiawan EB, Dunn EA, Andrews MH (2004) Fetal glucocorticoid exposure and hypothalamo-pituitary-adrenal (HPA) function after birth. Endocr Res 30:827-836.

McEwen BS (1987) Steroid hormones and brain development: some guidelines for understanding actions of pseudohormones and other toxic agents. Environ Health Perspect 74:177-184.

Meaney MJ, Szyf M, Seckl JR (2007) Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. Trends Mol Med 13:269-277.

Meijer OC, de Kloet ER (1998) Corticosterone and serotonergic neurotransmission in the hippocampus: functional implications of central corticosteroid receptor diversity. Crit Rev Neurobiol 12:1-20.

Mueller BR, Bale TL (2006) Impact of prenatal stress on long term body weight is dependent on timing and maternal sensitivity. Physiol Behav 88:605-614.

Murray L, Cooper PJ (1997) Postpartum depression and child development. Psychol Med 27:253-260.

Nomura Y, Wickramaratne PJ, Warner V, Mufson L, Weissman MM (2002) Family discord, parental depression, and psychopathology in offspring: ten-year follow-up. J Am Acad Child Adolesc Psychiatry 41:402-409.

Pavlovska-Teglia G, Stodulski G, Svendsen L, Dalton K, Hau J (1995) Effect of oral corticosterone administration on locomotor development of neonatal and juvenile rats. Exp Physiol 80:469-475.

Pawlby S, Hay DF, Sharp D, Waters CS, O'Keane V (2009) Antenatal depression predicts depression in adolescent offspring: prospective longitudinal community-based study. J Affect Disord 113:236-243.

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149-167.

Petropoulos S, Gibb W, Matthews SG (2010) Developmental expression of multidrug resistance phosphoglycoprotein (P-gp) in the mouse fetal brain and glucocorticoid regulation. Brain Res 1357:9-18.

Porsolt RD, Anton G, Blavet N, Jalfre M (1978) Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 47:379-391.

Renard GM, Rivarola MA, Suarez MM (2007) Sexual dimorphism in rats: effects of early maternal separation and variable chronic stress on pituitary-adrenal axis and behavior. Int J Dev Neurosci 25:373-379.

Roberts D, Dalziel S (2006) Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev 3:CD004454.

Sapolsky RM (1985) Glucocorticoid toxicity in the hippocampus: temporal aspects of neuronal vulnerability. Brain Res 359:300-305.

Sapolsky RM, Meaney MJ (1986) Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res 396:64-76.

Scheff SW, Morse JK, DeKosky ST (1988) Hydrocortisone differentially alters lesion-induced axon sprouting in male and female rats. Exp Neurol 100:237-241.

Seckl JR, Cleasby M, Nyirenda MJ (2000) Glucocorticoids, 11beta-hydroxysteroid dehydrogenase, and fetal programming. Kidney Int 57:1412-1417.

Secoli SR, Teixeira NA (1998) Chronic prenatal stress affects development and behavioral depression in rats. Stress 2:273-280.

Sloboda DM, Challis JR, Moss TJ, Newnham JP (2005) Synthetic glucocorticoids: antenatal administration and long-term implications. Curr Pharm Des 11:1459-1472.

Slotten HA, Kalinichev M, Hagan JJ, Marsden CA, Fone KC (2006) Long-lasting changes in behavioural and neuroendocrine indices in the rat following neonatal maternal separation: gender-dependent effects. Brain Res 1097:123-132.

Smith JW, Seckl JR, Evans AT, Costall B, Smythe JW (2004) Gestational stress induces post-partum depression-like behaviour and alters maternal care in rats. Psychoneuroendocrinology 29:227-244.

Stanton ME, Levine S (1990) Inhibition of infant glucocorticoid stress response: specific role of maternal cues. Dev Psychobiol 23:411-426.

Stoffel EC, Craft RM (2004) Ovarian hormone withdrawal-induced "depression" in female rats. Physiol Behav 83:505-513.

Stone EA, Egawa M, McEwen BS (1988) Novel effect of chronic corticosterone treatment on escape behavior in rats. Behav Neural Biol 50:120-125.

Talge NM, Neal C, Glover V (2007) Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? J Child Psychol Psychiatry 48:245-261.

Theogaraj E, John CD, Christian HC, Morris JF, Smith SF, Buckingham JC (2005) Perinatal glucocorticoid treatment produces molecular, functional, and morphological changes in the anterior pituitary gland of the adult male rat. Endocrinology 146:4804-4813.

Vicedomini JP, Nonneman AJ, DeKosky ST, Scheff SW (1986) Perinatal glucocorticoids disrupt learning: a sexually dimorphic response. Physiol Behav 36:145-149.

Wapner RJ, Sorokin Y, Mele L, Johnson F, Dudley DJ, Spong CY, Peaceman AM, Leveno KJ, Malone F, Caritis SN, Mercer B, Harper M, Rouse DJ, Thorp JM, Ramin S, Carpenter MW, Gabbe SG (2007) Long-term outcomes after repeat doses of antenatal corticosteroids. N Engl J Med 357:1190-1198.

Watson JB, Mednick SA, Huttunen M, Wang X (1999) Prenatal teratogens and the development of adult mental illness. Dev Psychopathol 11:457-466.

Wei L, David A, Duman RS, Anisman H, Kaffman A (2010) Early life stress increases anxiety-like behavior in Balbc mice despite a compensatory increase in levels of postnatal maternal care. Horm Behav 57:396-404.

Wilcoxon JS, Redei EE (2007) Maternal glucocorticoid deficit affects hypothalamic-pituitary-adrenal function and behavior of rat offspring. Horm Behav 51:321-327.

Xu Z, Zhang Y, Hou B, Gao Y, Wu Y, Zhang C (2010) Chronic corticosterone administration from adolescence through early adulthood attenuates depression-like behaviors in mice. J Affect Disord (Epub ahead of print).

Yeh KY (1984) Corticosterone concentrations in the serum and milk of lactating rats: parallel changes after induced stress. Endocrinology 115:1364-1370.

Young BJ, Cook CJ (2004) Stress-induced modification of anxiety in rats is dependent on reproductive status. Physiol Behav 80:569-575.

Zuena AR, Mairesse J, Casolini P, Cinque C, Alema GS, Morley-Fletcher S, Chiodi V, Spagnoli LG, Gradini R, Catalani A, Nicoletti F, Maccari S (2008) Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. PLoS One 3:e2170.

Figure Legends

Figure 1

Experiment Overview and Timeline.

Figure 2

Body weight after weaning (A) and in adulthood (B). A: Body weight of male and female offspring at postnatal day 23 (weaning). Males weighed significantly more than females. Post10, Preg40, Post40 and P+P40 pups weighed less than undisturbed or oil controls. B: Body weight of male and female offspring in adulthood. Males were significantly heavier than females. All low CORT groups weighed significantly less than oil controls. However, oil controls weighed more than undisturbed controls. a: significantly different from undisturbed controls; b: significantly different from oil controls; $p<0.05$.

Figure 3

Percentage of time spent with each behavior (struggling, swimming and immobile) during the Forced Swim Test. Offspring from the Preg40 group showed increased immobility and decreased swimming compared to undisturbed or oil offspring. a: significantly different from undisturbed controls; b: significantly different from oil controls; $p<0.05$.

Figure 4

Peripheral distance travelled in the Open Field Test.

Females were significantly more active in the Open Filed Test than males. Female oil covered more distance than female undisturbed controls ($p=0.01$) and Post10 females travelled

significantly less (p=0.004), while P+P10 females travelled significantly more than oil controls (p=0.009). Similarly, for males, P+P10 travelled significantly more peripheral distance compared to male oil controls (p=0.05), but Preg40 travelled significantly less than oil controls (p=0.02) or undisturbed controls (p=0.01).a: significantly different from undisturbed controls; b: significantly different from oil controls

Figure 5

Behavior in the Elevated Plus Maze.

Males exposed to high CORT during the postpartum (i.e. Post40 and P+P40) showed more anxiety-like behavior in the Elevated Plus Maze by spending more time in the closed arm and revealing a higher closed/open & center ratio. a: significantly different from undisturbed controls; b: significantly different from oil controls; $p<0.05$.

Figure 6

Total time it took to capture the animals and the cumulative capture score based on a 7-point scale for resistance to capture during approach, touch and lift up. The P+P10 group was captured faster compared to oil controls, while the Preg40 group took longer to be captured. All high CORT groups had higher cumulative scores compared to oil or undisturbed controls. a: significantly different from undisturbed controls; b: significantly different from oil controls; $p<0.05$.

Figure 7

Corticosterone (CORT) serum levels during restraint stress, measured before the stressor (t0, baseline), during the restraint stress (t30), at the end of the stressor (t60) and an hour after return

to the home cage (t120, recovery). Animals that were exposed to CORT *in utero* alone (Preg10 and Preg40) had lower baseline levels compared to undisturbed controls, while animals exposed to low CORT during the postpartum (Post10 and P+P10) had higher baseline levels compared to oil controls, which was particularly obvious in females. One hour after the stress, all high CORT groups had significantly lower CORT levels compared to undisturbed controls, again most evident in females, while the P+P10 groups had also higher recovery CORT levels compared to oil and undisturbed controls. a: significantly different from undisturbed controls; b: significantly different from oil controls; $p<0.05$.

Table 1

Table 1 Mean (± SEM) weight per pup during the postpartum period [grams]

	PND1	PND9	PND13	PND17	PND20
Undisturbed	6.9 ± 0.1	22.2 ± 1.1	33.1 ± 1.3	40.9 ± 1.9	47.1 ± 3.0
Oil	6.7 ± 0.2	21.6 ± 0.4	31.0 ± 0.8	39.6 ± 0.8	49.3 ± 0.9
Preg10	6.7 ± 0.1	22.3 ± 0.8	32.0 ± 0.9	38.1 ± 2.3 [a]	50.4 ± 2.3 [a]
Post10	7.0 ± 0.3	20.3 ± 0.4	29.3 ± 0.5 [a]	35.2 ± 0.9 [a,b]	44.2 ± 1.3 [a,b]
P+P10	6.7 ± 0.2	22.0 ± 0.8	31.0 ± 1.2	39.0 ± 1.2	48.2 ± 2.2
Preg40	6.5 ± 0.4	20.0 ± 0.9	28.3 ± 1.2 [a,b]	36.7 ± 1.6 [a,b]	45.0 ± 2.0 [b]
Post40	6.7 ± 0.2	19.9 ± 0.2	27.6 ± 0.2 [a,b]	35.1 ± 0.6 [a,b]	45.2 ± 0.7 [b]
P+P40	6.0 ± 0.3	17.8 ± 0.9 [a,b]	24.2 ± 1.1 [a,b]	30.4 ± 1.5 [a,b]	38.0 ± 1.9 [a,b]

[a] significantly different ($p<0.05$) from Undisturbed pups, [b] significantly different ($p<0.05$) from Oil pups

Table 2

Table 2 Open Field Test Measures (Mean ±S.E.M.)

Group	# of rears	Fecal boli	Center entering latency [s]	Time in center [s]	Center distance [m]	Center /total distance
			Males*			
Undisturbed	45.5 (± 4.8)	1.9 (± 0.7)	96.5 (± 67.3)	9.5 (± 3.2)	1.6 (± 0.6)	.025 (±.009)
Oil	40.8 (± 4.2)	1.1 (± 0.4)	112.9 (± 37.5)	17.6 (± 2.3)	3.6 (± 0.4)	.058 (±.011)
Preg10	48.8 (± 7.0)	3.4 (± 0.8)	138.0 (± 68.8)	14.6 (± 4.5)	3.0 (± 1.0)	.042 (±.012)
Post10	45.3 (± 3.0)	3.0 (± 0.7)	101.6 (± 16.0)	18.1 (± 2.3)	3.6 (± 0.5)	.057 (±.008)
P+P10	41.9 (± 5.6)	2.5 (± 0.8)	118.6 (± 47.3)	13.0 (± 2.9)	2.7 (± 0.5)	.040 (±.007)
Preg40	36.8 (± 3.4)	2.1 (± 1.0)	48.1 (± 10.1)	11.4 (± 2.2)	2.0 (± 0.4)	.043 (±.007)
Post40	48.0 (± 8.4)	3.3 (± 1.2)	45.1 (± 9.6)	17.4 (± 4.7)	3.6 (± 0.8)	.057 (±.011)
P+P40	44.1 (± 5.6)	1.6 (± 0.7)	78.3 (± 32.2)	15.0 (± 3.6)	3.3 (± 0.8)	.055 (±.014)
			Females			
Undisturbed	45.8 (± 3.5)	1.3 (± 0.9)	61.4 (± 40.1)	21.9 (± 5.5)	4.2 (± 0.8)	.065 (±.013)
Oil	56.8 (± 4.3)	0.6 (± 0.4)	48.4 (± 11.7)	22.8 (± 2.4)	5.1 (± 0.5)	.070 (±.006)
Preg10	48.7 (± 6.5)	0.6 (± 0.4)	70.0 (± 23.5)	17.5 (± 1.6)	3.8 (± 0.5)	.052 (±.009)
Post10	43.6 (± 2.3)	0.4 (± 0.4)	24.5 (± 5.7)	25.5 (± 4.2)	5.5 (± 0.8)	.080 (±.012)
P+P10	51.4 (± 4.3)	0.4 (± 0.3)	66.3 (± 15.9)	30.7 (± 4.0)	5.9 (± 0.9)	.072 (±.010)
Preg40	51.9 (± 3.2)	0.3 (± 0.3)	68.1 (± 18.6)	20.8 (± 4.5)	5.1 (± 1.1)	.068 (±.013)
Post40	52.0 (± 1.7)	1.1 (± 0.7)	37.6 (± 16.6)	18.8 (± 2.8)	4.4 (± 0.5)	.063 (±.006)
P+P40	54.0 (± 5.2)	0.0 (± 0.0)	47.5 (± 22.2)	16.0 (± 3.6)	3.6 (± 0.8)	.057 (±.013)

* Males showed significantly fewer rears ($p=0.01$), more fecal boli ($p<0.001$), higher latency to enter the center ($p=0.02$) and less time and distance in the center ($p<0.001$) than females.

Table 3

Table 3 Entries into and time spent in closed or open arms or center during the Elevated Plus Maze (EPM)

	Entries		Time	
Group	Closed arm	Open arm	Open arm	Center
Undisturbed	10.5 ± 0.8	1.6 ± 0.3	17.3 ± 2.6	82.0 ± 8.0
Oil	11.2 ± 0.5	2.1 ± 0.4	19.5 ± 3.8	81.1 ± 4.2
Preg10*	9.6 ± 0.8	0.9 ± 0.2	6.8 ± 1.8	89.8 ± 8.8
Post10*	9.1 ± 0.6	1.6 ± 0.9	13.4 ± 3.3	67.7 ± 6.4
P+P10	10.3 ± 0.6	1.4 ± 0.8	11.4 ± 2.1	106.1 ± 6.5
Preg40	12.5 ± 0.8	2.2 ± 1.3	13.6 ± 3.3	80.6 ± 5.2
Post40	10.6 ± 0.9	1.3 ± 0.3	7.0 ± 2.5	65.7 ± 7.4
P+P40	11.6 ± 1.1	2.0 ± 0.8	12.1 ± 3.8	70.5 ± 7.0

*Preg10 and Post10 animals made significantly fewer overall entries compared to oil rats (p's < 0.02).

Table 4

Table 4 Summary of selected effects per group compared to **oil** controls

	Preg10	Post10	P+P10	**Preg40**	**Post40**	**P+P40**
Weight						
At weaning		↓		↓	↓	↓
As adults[>a]	↓	↓	↓			
Forced Swim Test						
Immobility				↑		
Open Field Test						
Peripheral distance [F:>a]		F: ↓	↑	M:↓		
Elevated Plus Maze						
Time in closed arm					↑	M:↑
Closed / Open ratio					M:↑	M:↑
Resistance to capture						
Time to capture			↓	↑		
Capture Score				↑	↑	↑
Restraint stress[#]						
Baseline	↓[#]	↑	↑	↓[#]		
End of stress	↑		↑	↑		
Recovery			↑	↓[#]	↓[#]	↓[#]

>a: indicates that oil controls were significantly higher compared undisturbed controls

#: for restraint stress differences are also displayed if the group was different compared to undisturbed controls; F: only in Females; M: only in Males

Figure 1

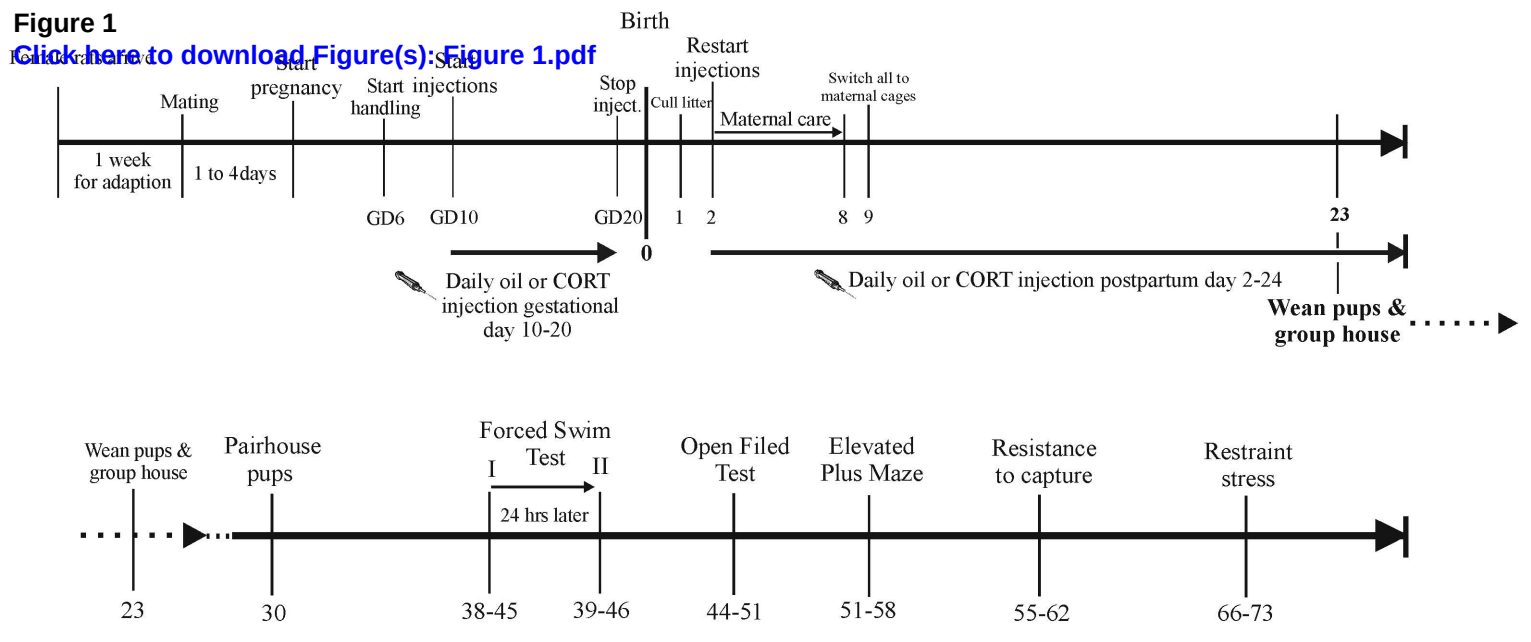

Figure 2

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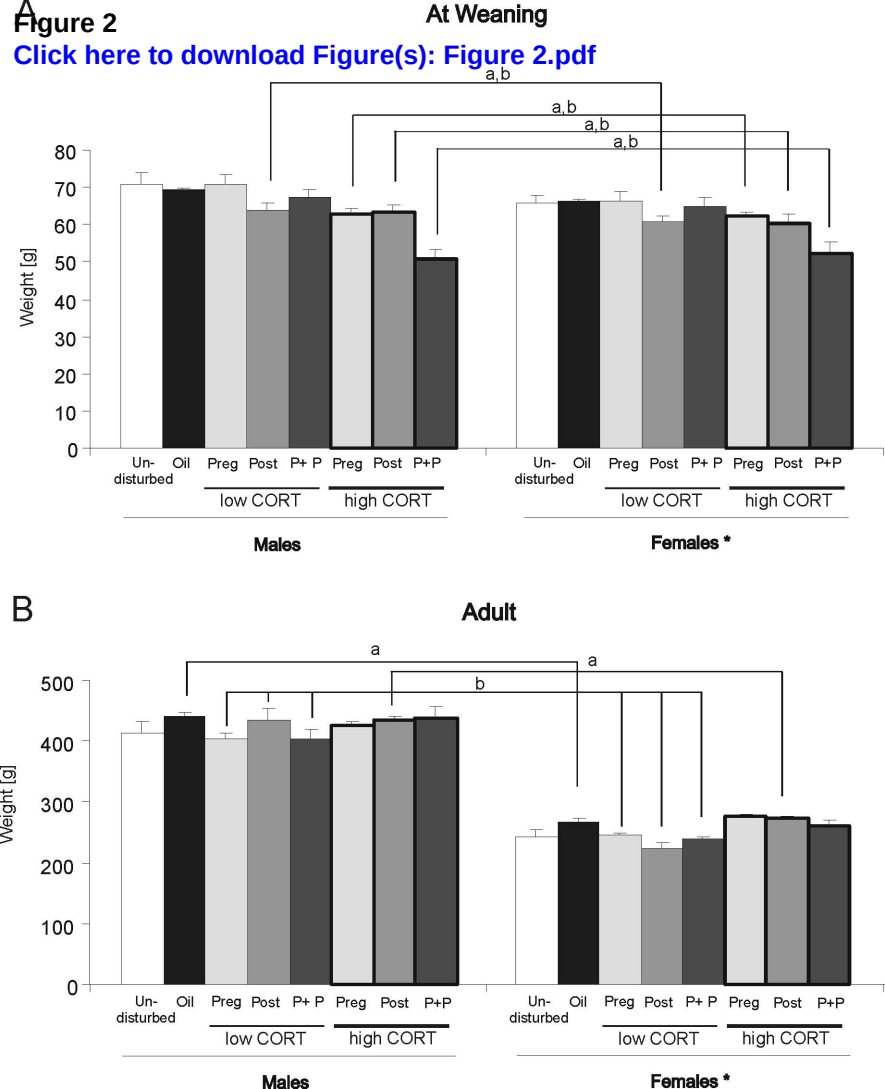

Figure 3

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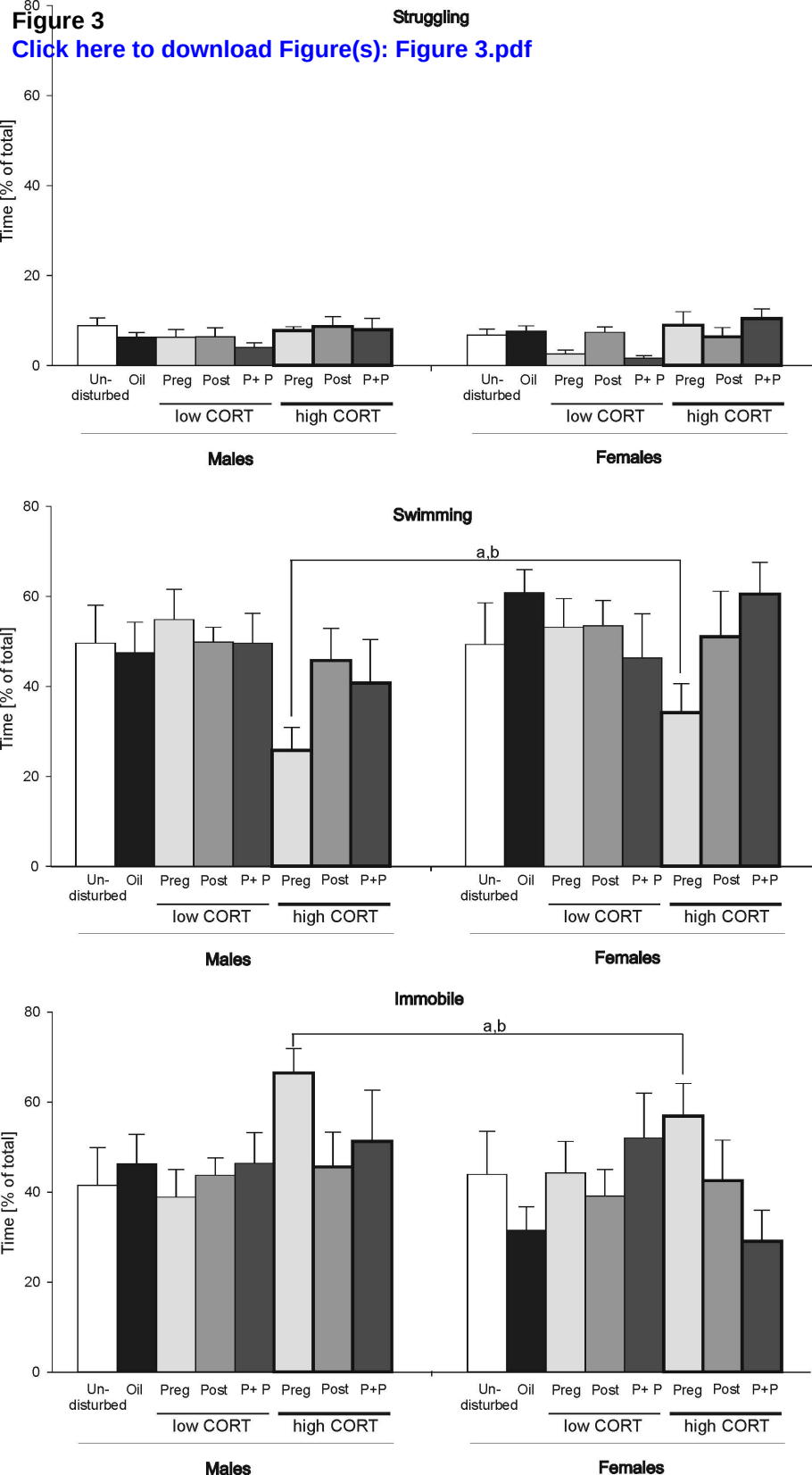

Figure 4

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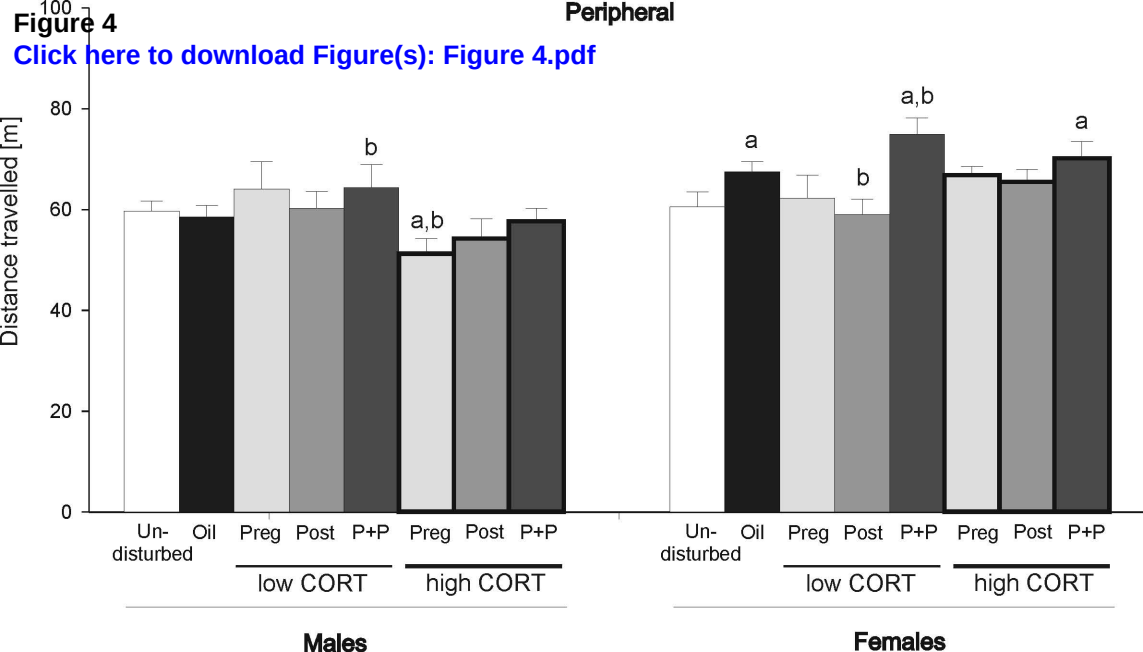

Figure 5

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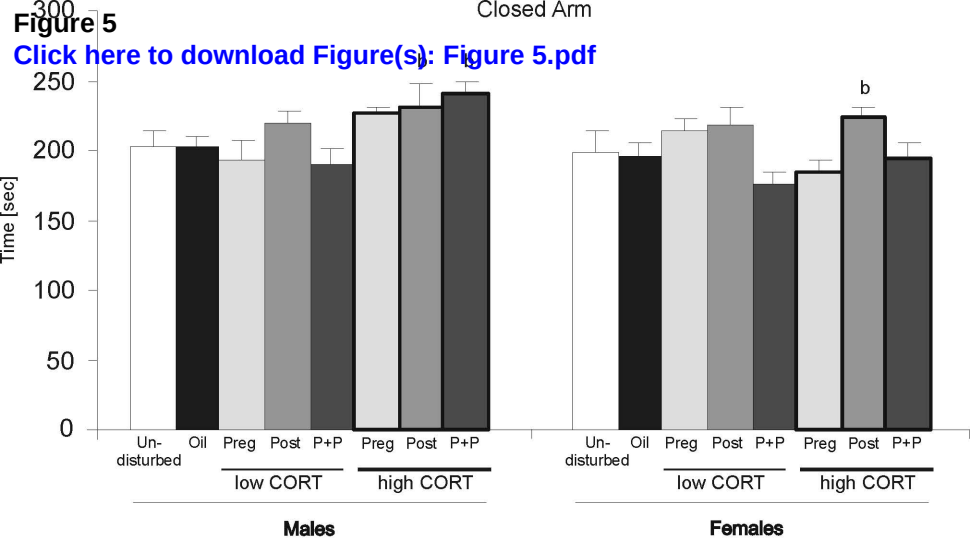

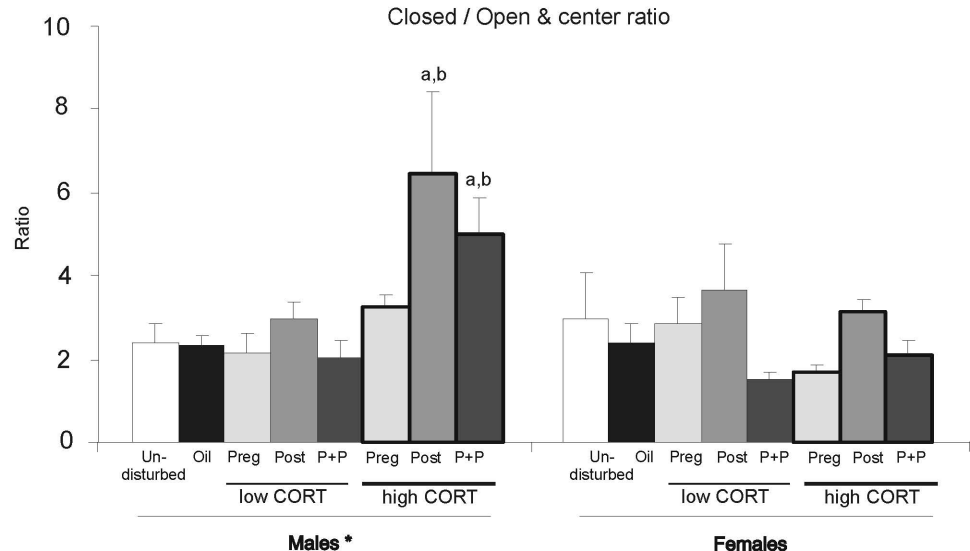

Figure 6

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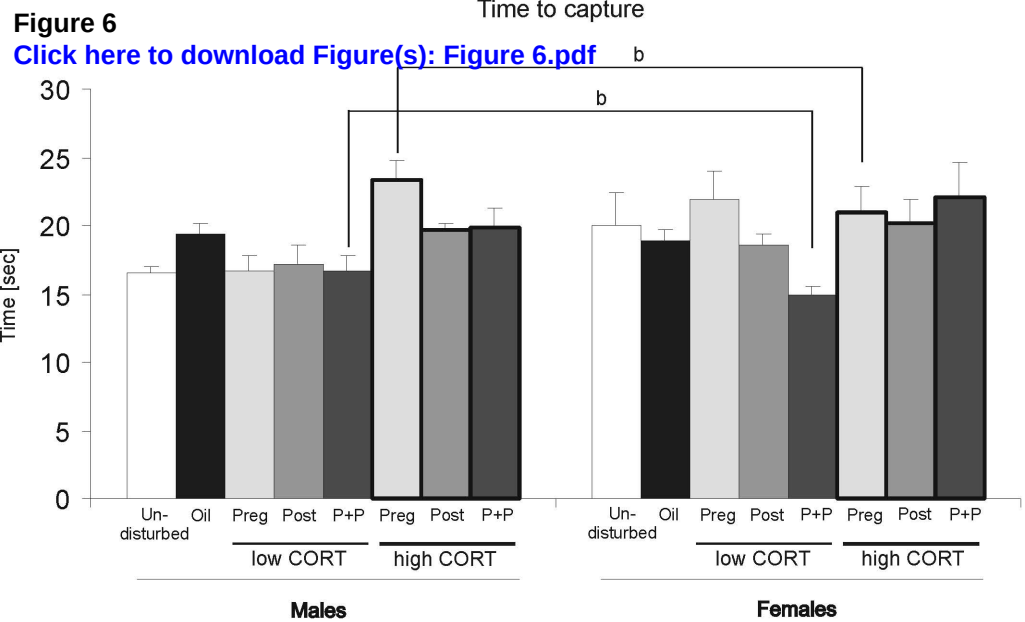


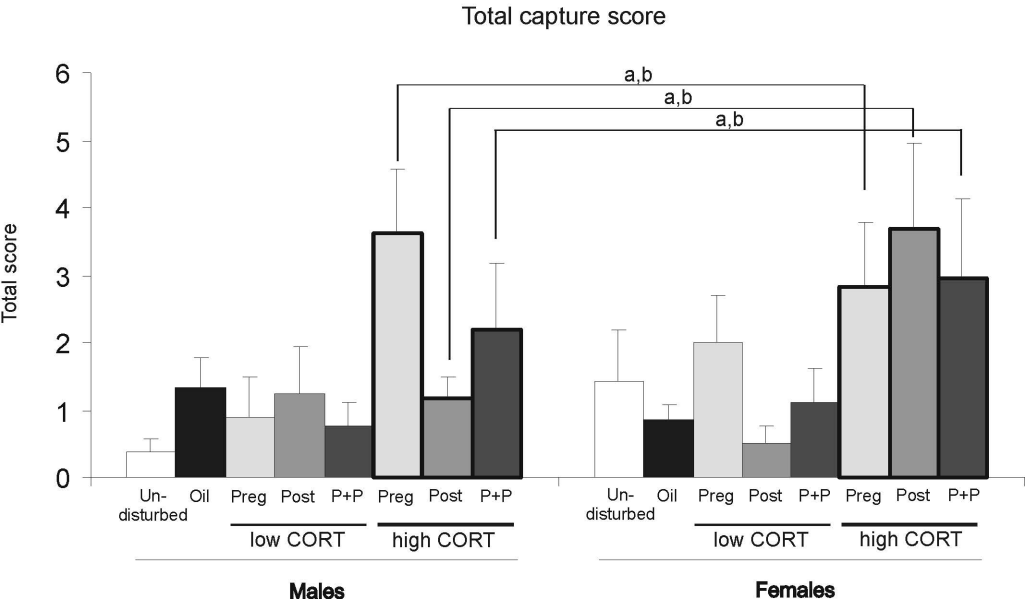
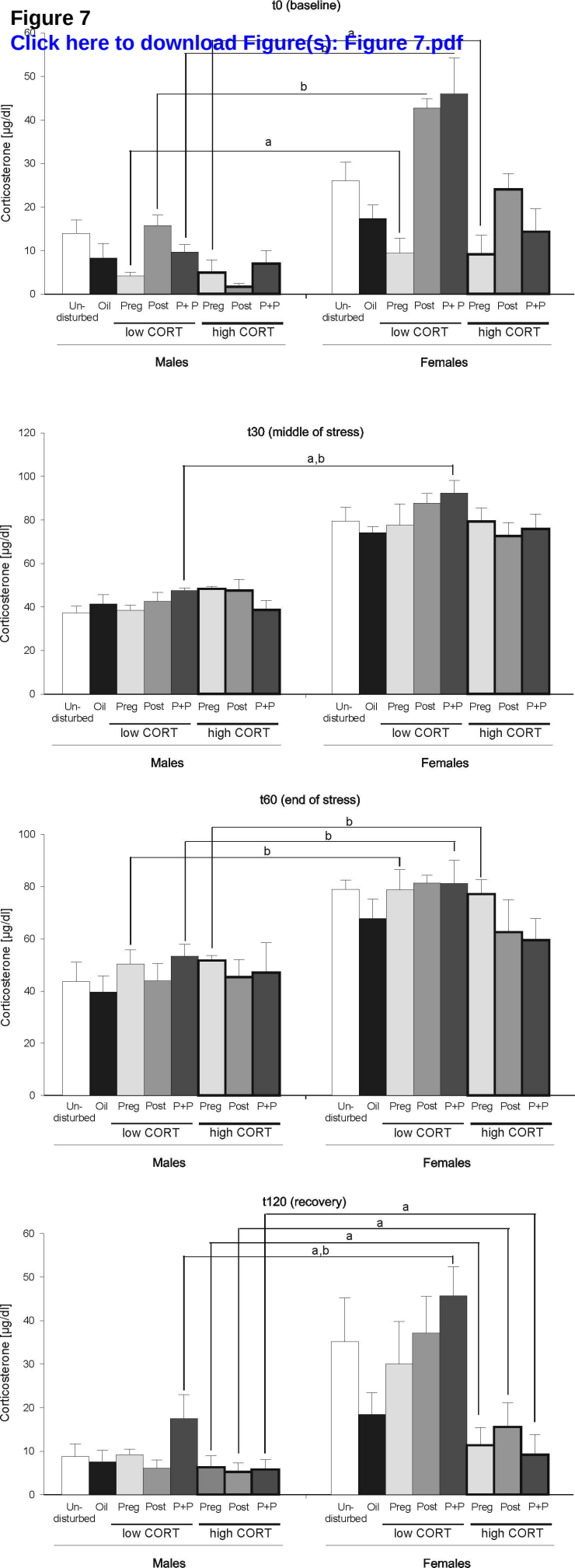


Figure 7



Supplementary Table 1

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Supplementary Table 2

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Supplementary Table 3

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