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Maternal Glucocorticoid Deficit Affects Hypothalamic-Pituitary-Adrenal Function and Behavior of Rat Offspring

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Abstract

Detrimental consequences of prenatal stress include increased hypothalamic-pituitary-adrenal (HPA) function, anxiety and depression-like behavior in adult offspring. To identify the role of maternal corticosterone milieu in the fetal programming of adult function, we measured these same behavioral and hormonal endpoints after maternal adrenalectomy (ADX) and replacement with normal or moderately high levels of corticosterone (CORT). Adult male and female offspring exhibited differing HPA responses to maternal ADX. In female offspring of ADX mothers, exaggerated plasma ACTH stress responses were reversed by the higher, but not the lower, dose of maternal CORT. In contrast, male offspring of both ADX and ADX dams with higher CORT replacement showed exaggerated ACTH stress responses. Hypothalamic glucocorticoid receptor (GR) expression was decreased in these latter groups, while hippocampal GR increased only in the ADX offspring. Activity of young offspring of ADX dams replaced with the higher dose of CORT decreased in the open field test of exploration/anxiety, while immobility behavior of adult offspring in the forced swim test of depression increased following maternal ADX or higher levels of CORT replacement. Interestingly, for some measures, none or moderately high CORT replacement resulted in similar deficits in this study. These findings are in accord with consequences of prenatal stress or prenatal dexamethasone exposure, suggesting that a common mechanism may underlie the effects of too low or too high maternal glucocorticoids on adult HPA function and behavior.

Keywords

stress; maternal adrenalectomy; glucocorticoid receptor; birth weight

Introduction

Glucocorticoids are essential for the development of various organ systems (Meyer, 1985), and the ability of the prenatal glucocorticoid milieu to program the hypothalamic-pituitary-adrenal (HPA) axis has been demonstrated in several species (Matthews, 2002). Glucocorticoid administration during pregnancy is a common treatment to promote lung maturation in fetuses at risk of being premature, albeit the known risk that prenatal exposure to glucocorticoids during a critical period of brain development can permanently alter HPA function (Matthews et al, 2002). In rats, elevated prenatal glucocorticoid levels are associated with growth

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retardation, sexually dimorphic alterations in HPA regulation of the offspring, and programming of other physiological systems (Benediktsson et al, 1993; Cleasby et al, 2003).

Previous studies conducted to determine the role of maternal glucocorticoids in the programming of the HPA function of the adult convincingly indicate that increased transfer of glucocorticoids from the mother to the fetus is the key factor in this prenatal imprinting (Barbazanges et al, 1996; Seckl, 2000). Similarly, prenatal stress and is known to result in increased anxiety and depression-like behavior in the offspring (Weinstock, 1997, 2001, 2005) and excess maternal stress-induced corticosterone (CORT) predisposes the offspring to gender-specific alterations in behavior (Zagron and Weinstock, 2006). However, further studies are necessary to determine the mechanism by which decreased maternal glucocorticoids program adult offspring behavior.

This other extreme in the prenatal glucocorticoid milieu is represented by maternal Addison's disease, or any other cause of maternal hypocortisolemia. These decreased levels of maternal cortisol during pregnancy may cause intrauterine fetal growth retardation, leading to reduced birth weight (O'Shaughnessy and Hackett, 1984; Hilden and Ronnike, 1971) just as elevated maternal glucocorticoids do. A recent study suggests that pregnant women who develop posttraumatic stress disorder have lower cortisol levels than controls and their infants have greater distress to novelty (Brand et al., 2006). In animals, the removal of maternal glucocorticoids by adrenalectomy (ADX) leads to consequences in the offspring, some of which are similar to those observed in offspring exposed to elevated maternal glucocorticoids in utero. For example, newborn offspring of ADX mothers have reduced body weight (Smith et al, 1975; Wilcoxon et al, 2003), and adult offspring of ADX mothers tend to have higher resting plasma CORT values than controls (Milkovic et al, 1976). As increased and decreased maternal glucocorticoids may program some of the same physiological functions of the developing fetus in a biphasic manner, the question arose whether maternal or fetal glucocorticoids program the prenatal, and subsequently the adult, HPA axis.

Studies on the ontogeny of placental 11 β -HSD2 show that this "barrier" to maternal glucocorticoids is highest during mid-gestation and begins to decline during the last week of gestation (Waddell et al, 1998; Brown et al, 1996), when the fetal pituitary-adrenal system starts functioning in rats (Dubois and Hemming, 1991). Prior to embryonic day 18 (E18), CORT can cross the placenta, regulated by 11 β -HSD2, from the maternal to the fetal circulation (Dupouy, 1980) and influence the development of the fetal HPA axis as well as fetal brain function. However, the passage of CORT from the fetal to the maternal circulation seems to begin around E18, since after this time there is a transfer of fetal corticosterone to the adrenalectomized mother (Cohen et al., 1990; Sinha et al, 1997; Slone-Wilcoxon and Redei, 2004). Thus, fetuses of ADX dams are exposed to low CORT prior to E18, but high CORT of fetal origin after E18. Consistent CORT replacement to ADX dams just below and just above control CORT levels, therefore, can alter fetal CORT production and may indicate how tight the maternal-fetal pituitary-adrenal system is regulated.

Materials and Methods

Animals

All animal procedures were approved by the Northwestern University Animal Care and Use Committee. Adult male and female Sprague Dawley rats (viral free, 56–68 days of age; Harlan, Indianapolis, IN) were housed individually in a temperature and humidity controlled vivarium with regular light-dark cycles (light on at 700h and light off at 1900h). After seven days of acclimatization, rats were mated by placing a female in the male cage overnight. Mating was confirmed by microscopic analysis of vaginal smears for the presence of sperm the next morning. The day sperm was found was designated as gestational day 1.

Pregnant rats were assigned to four experimental groups. On gestational day 8, the rats received adrenalectomy (ADX) or sham-adrenalectomy (SHAM, n=5), performed dorsally under anesthesia (ketamine/xylazine 87/10 mg/kg BW). At the time of surgery the ADX dams received a placebo (ADX, n=5), 50 mg (ADX+CORT50, n=5) or 75 mg (ADX+CORT75, n=5), 21-day release corticosterone (CORT) pellet (Innovative Research of America, Sarasota, FL). The 50 mg CORT pellet produces values comparable to resting basal values, which may be lower in a pregnant rat with a larger extracellular volume (Diaz et al, 1995), and the 75 mg pellet produces constant CORT levels, which are slightly higher than resting basal levels (Spinedi and Gaillard, 1998). The drinking water of ADX dams was supplemented with 0.9% NaCl.

On postnatal day 1 between 800 and 1200h within six hours of delivery, trunk blood was collected from the mother and one male and one female from each litter for CORT determination. The average litter size was 10.2 $\pm$ 2.3, with no significant difference among the groups. All of the remaining pups, including SHAM offspring, were then fostered to non-ADX control dams as the stress of surgery may affect maternal behavior (Patin et al, 2002). At 21 days of age, pups were weaned and 3–4 animals of the same sex were housed by prenatal treatment. Following behavioral testing, animals were marked with ear tags and returned to their original groups.

At 21 days of age, 1–2 pups/sex/litter were tested in the open field test to measure activity/exploratory behavior. Between 60 and 70 days of age, adult offspring (2–3 animals/sex/litter) were exposed to the forced swim test to assess depressive behavior. All testing took place between 1100 and 1500 h. At 80–90 days of age, animals underwent a restraint stress experiment as described below.

Open Field Test (OFT)

The OFT apparatus is a circular arena divided into a center circle and a peripheral circle with a total diameter of 60cm and height of 30cm. The illuminance in the open field arena was approximately 160 lux. Animals were placed in the center of the open field and remained there for ten minutes. The OFT was cleaned with 50% alcohol between animals. Using a video image-based computer “tracking” system (Limelight from Actimetrics, Evanston, IL), the following measures were recorded: total time in outer circle, total time in inner circle and total distance traveled. Latency, rearing and defecation were scored manually and also recorded.

Forced Swim Test (FST)

The FST procedure used was described previously (Solberg et al, 2003). Briefly, animals were placed into a glass cylinder (30 cm diameter, 45 cm deep) of 25°C tap water for 15 min. Twenty four hours later rats were again placed into the cylinder of water for 5 min. Activity during the second swim test was video-recorded for subsequent scoring using a time-sampling technique previously described (Detke et al, 1995) in which behavior was scored as immobility, climbing, or swimming every 5 sec. We have previously shown that immobility in the FST is not related to body weight (Solberg et al, 2003).

Restraint Stress

At 80–90 days of age, adult male and female rats from each prenatal treatment group were exposed to a 30-minute restraint stress between 1000–1200h. The animals were individually caged the evening before the restrain stress experiment and the next day restrained in a plastic flat-bottom rodent restrainer (Harvard Apparatus). Animals were killed by decapitation prior to stress (basal, t=0), and 30, 45 and 60 minutes after the onset of the 30 min stress. At least one, but at most 2 male and female offspring from each litter were used for each time point. Trunk blood was collected into chilled tubes containing EDTA (8 μ l/ml whole blood), and kept

frozen (-80°C) in aliquots. Brains of animals that did not undergo the restrain stress ($t=0$) were also collected and kept at -80°C .

Radioimmunoassays

ACTH immunoreactivity was measured as previously described (Redei et al, 1993) using [^{125}I]ACTH (1–39) (Amersham, Piscataway, NJ). The assay sensitivity was 6.7 pg/ml. The intra- and interassay coefficients of variation were 7.6 and 8.5%, respectively.

CORT concentrations were measured as described previously (Slone and Redei, 2002) in unextracted plasma using [^{125}I] CORT RIA (ICN Biomedicals, Carson, CA.). CORT antiserum cross-reactivity is less than 0.3% with other steroids. The assay sensitivity was 9 pg/ml, while the intra- and interassay coefficients of variation were 6.1 and 8.7%, respectively.

RNA Isolation and Northern Analysis

Basal rat brains were rapidly dissected, as described previously (Wilcoxon et al, 2005), on ice and immediately placed on dry ice. Briefly, the rat brain matrix was used to prepare the primary sections, and specific brain regions then were dissected under microscope according the Paxinos coordinates (Paxinos and Watson, 1997): amygdala (AP -0.58 to -2.18 , ML 1.5 to 4.5, DV 4 to 5.75), prefrontal cortex (AP 5.20 to 1.70, ML 0 to 3.3, DV 9.0 to 4.4), hypothalamus (AP -0.30 to -4.16 , ML 0 to 2.2, DV -0.40 to 2.8) and hippocampus (AP -2.12 to -6.0 , ML 0 to 5.0, DV 5.4 to 7.6). Tissues were stored at -80°C .

Extraction of total RNA was performed using Trizol reagent, according to the manufacturer's protocol (Life Technologies, Grand Island, NY). The quality and quantity of RNA were analyzed by gel electrophoresis and spectrophotometry. For northern analysis, 8–10 μg of RNA from each sample was electrophoresed on a 1% agarose-formaldehyde gel, blotted onto a nitrocellulose filter, and fixed by UV-crosslinking as described previously (Redei et al, 1993). The plasmid containing the β -actin cDNA probe was kindly provided by Dr. Michael Prystowsky, Albert Einstein University. The GR probe was generated by PCR using primers specific for the rat glucocorticoid receptor cDNA (5'-TGCAGCAGTGAAATGGGCAA-3'; 5'-GGGAATTCAATACTCATGGTC-3'). The generated GR probe was 533bp in length. Probes were labeled with [α - ^{32}P]dCTP by random primer labeling (Feinberg and Vogelstein, 1983) using the Random Primers DNA Labeling System kit (Life Technologies, Grand Island, NY). Filters were hybridized with cDNA probes overnight at 42°C in ULTRAhyb hybridization buffer (Ambion, Austin, TX) following the manufacturer's protocol. Filters were washed twice for 15 min each in 2X SSC/0.1% sodium dodecyl sulfate (SDS) at 42°C , twice for 30 min each in 0.1X SSC/0.1% SDS at 42°C , and exposed to Hyperfilm MP autoradiography film (Amersham Pharmacia Biotech, Piscataway, NJ) at -80°C with intensifying screens. Autoradiographs were scanned and analyzed using NIH Image (Wayne Rasband, NIH, Bethesda, MD). Specific mRNA levels were normalized to the β -actin mRNA level of each sample.

Statistics

The data were analyzed by ANOVA, one-way design for GR analysis after determining the lack of sex effect, a two-factor design for behavioral measures (sex, maternal treatment) and a three-factor design for hormone measures (sex, maternal treatment, time). Litter was a nested factor. The Tukey HSD test, with a $p<0.05$, was used as a post-hoc test to locate significant differences among groups, when appropriate. F statistics are reported in the results section and post-hoc statistics are indicated in the figures. NCSS software (Kaysville, UT) was used for all analyses.

Results

Maternal and Neonatal Corticosterone Levels

Inverse relationships between plasma CORT levels of ADX and ADX+CORT50 mothers and their neonates are shown in Table 1. In contrast, ADX+CORT75 mothers had significantly increased plasma CORT values compared to SHAM mothers, while their neonates had plasma CORT levels identical to those of SHAM controls. Similar CORT data have been described previously (Slone-Wilcoxon and Redei, 2004), but reported here to highlight the maternal-fetal glucocorticoid milieu.

Body Weight and HPA Function in Adult Offspring

Adult male and female offspring of ADX+CORT75 dams weighed significantly ($p<0.05$) more than any other offspring (maternal treatment: $F[3,131]=4.3$; $p<0.01$; Figure 1), and as expected, males weighed more than females overall (sex: $F[1,131]=267.94$; $p<0.01$).

Maternal adrenalectomy and CORT replacement affected ACTH responses to stress differently in male and female offspring, with males having generally higher ACTH levels than females (treatment: $F[3,128]=2.76$; $p<0.05$; sex: $F[1,128]=21.48$; $p<0.01$; sex $\times$ treatment $F[3,128]=2.53$, $p<0.05$). The profile of the ACTH response to stress differed by maternal glucocorticoid milieu and sex (time: $F[3,128]=62.68$, $p<0.01$; sex $\times$ treatment $\times$ time: $F[9,128]=2.4$, $p<0.05$). The sexually dimorphic effect of differing maternal glucocorticoid milieu is evident as only the ADX+CORT50 female offspring had exaggerated ($p<0.05$) ACTH stress responses to the 30-minute restraint stress (Figure 2a), while both the ADX and ADX+CORT75 male offspring had significantly ($p<0.05$) elevated ACTH stress responses at this time point (Figure 2b).

In contrast, there were no significant effects of maternal adrenalectomy and CORT replacement on plasma CORT responses to stress, although there were significant sex differences in this measure (sex: $F[1,143]=6.24$; $p<0.01$). The profile of the CORT response to stress differed by sex, but not by maternal glucocorticoid milieu (time: $F[3,143]=107.9$, $p<0.001$; sex $\times$ time: $F[3,143]=2.84$, $p<0.05$). This sex difference is most visible at the 30 min time point where females had significantly ($p<0.05$) elevated plasma CORT levels compared to males (Figures 2a and b), while plasma CORT levels were not different between the sexes 45 min after the onset of stress.

GR mRNA Expression in Adult Brain

No sex differences were found in GR mRNA levels in any of the brain regions, so male and female data were pooled. There were no significant differences in GR expression (normalized to β -actin mRNA levels) between the treatment groups in the frontal cortex (SHAM: $1.16 \pm .19$; ADX: $1.24 \pm .2$; ADX+CORT50: $1.04 \pm .15$; ADX+CORT75: $0.82 \pm .24$) or amygdala (SHAM: $1.11 \pm .2$; ADX: $1.16 \pm .21$; ADX+CORT50: $0.981 \pm .18$; ADX+CORT75: $1.06 \pm .11$). Significant differences in GR mRNA levels were found in the hippocampus (treatment: $F[3,36]=4.55$; $p<0.05$) and the hypothalamus (treatment: $F[3,36]=4.6$; $p<0.05$) of offspring with various prenatal treatments (Figure 3). GR expression increased significantly ($p<0.05$) in the hippocampus of ADX adult offspring compared to SHAM controls, and this increased expression was normalized by CORT replacement to the mother. In the hypothalamus, GR mRNA levels were lower in both ADX and ADX+CORT75 offspring ($p<0.05$) compared to SHAM offspring, indicating a biphasic response pattern in response to maternal glucocorticoids.

Behavior in the OFT and the FST

Maternal treatment altered center time in both male and female offspring (treatment: $F[3,62]=7.15$; $p<0.01$, Figure 4A) with no significant interaction between sex and treatment. The main

effect of treatment was seen specifically in ADX+CORT50 and ADX+CORT75 offspring, who spent less time in the center of the open field compared to SHAM offspring (Figure 4A).

In general, males were less active than females, as measured by total distance traveled in the OFT arena, and maternal treatment altered activity similarly in both males and females (sex: $F[1,62]=5.58$; $p<0.05$, treatment: $F[3,62]=4.31$; $p<0.05$, Figure 4B). Particularly, ADX+CORT75 offspring showed significantly ($p<0.05$) decreased activity compared to those of SHAM (Figure 4B). Other measures, including latency to leave the center, defecation or rearing, did not differ among the treatment groups (data not shown).

In the forced swim test, adult males floated less than females (sex: $F[1,91]=7.85$, $p<0.05$; Figure 4C). A major effect of maternal treatment on floating occurs in females (sex x treatment: $F[3,91]=2.94$, $p<0.05$); female offspring of ADX and ADX+CORT75 dams remained immobile significantly longer than females of SHAM or ADX+CORT50 dams. In contrast, maternal treatment had no effect on male immobility. Swimming behavior shows the exact opposite of immobility (Females: SHAM 56.3 ± 2.3 ; ADX 52.7 ± 1.8 ; ADX+CORT50 57.7 ± 2.4 ; ADX+CORT75 51.7 ± 1.5 ; Males: SHAM 57.8 ± 2.1 ; ADX 56.5 ± 2.9 ; ADX+CORT50 58.2 ± 2.0 ; ADX+CORT75 56.9 ± 2.6). This active behavior in the FST is significantly less in females than males and offspring of ADX and ADX+CORT75 dams swim less than those of ADX+CORT50 (sex: $F=18.96$, $p<0.001$; treatment: $F=5.06$, $p<0.01$; ADX+CORT50 vs ADX and ADX+CORT75, $p<0.05$). The sex and treatment interaction did not reach significance ($F=2.29$; $p=0.08$), but the trend supports the female-specific effect observed in floating.

Discussion

In the present study, we found that decreased levels of maternal glucocorticoids during the second and third week of pregnancy alter adult body weight, plasma ACTH stress responses and selected anxiety, depression-like behavioral measures in the offspring.

Evidence from the human and animal literature suggests that adverse fetal environment permanently programs physiology (Barker, 1990), leading to increased risk of several disorders including metabolic, neuroendocrine and psychiatric illnesses. Prenatal exposure to environmental stressors, such as famine, leads to impaired metabolic function (de Rooij et al., 2006) and increased prevalence of obesity in humans (Roseboom et al., 2006). Similarly, prenatal exposure to elevated glucocorticoids is known to result in low birth weight and impaired metabolic function in adulthood (Seckl and Meaney, 2004). Interestingly, birth weight is also low of offspring of ADX mothers (Smith et al, 1975; Wilcoxon et al, 2003), and ADX newborns from this study also had significantly reduced birth weight as previously published (Slone-Wilcoxon and Redei, 2004). In contrast, offspring of ADX+CORT75 mothers, who had normal birth weights, showed increased body weight as adults. A similarly increased body weight has been described as a consequence of stress during pregnancy in mice (Mueller and Bale, 2006). The increased body weight observed in our adult offspring exposed to greater than control CORT levels during pregnancy could be a potential model for fetal origin of obesity via alterations in metabolic pathways, similar to those described for undernutrition (Vickers et al., 2000).

An interesting finding in this study is the discordant alteration of ACTH stress responsiveness between male and female ADX offspring. Male offspring of ADX mothers demonstrated significantly elevated peak ACTH, but not CORT, stress responses. In contrast, female offspring of ADX dams had no alteration in their peak ACTH responses, but had significantly lower ACTH levels at 45 min post-stress. Previous studies have found prenatally stressed males have lower, while females have higher, ACTH responses to stress compared to controls

(Petropoulos and Lau, 1973; Barbazanges et al, 1996). These data are opposite of present findings in ADX offspring.

The mechanism, by which sex differences emerge following alterations in prenatal glucocorticoid milieu, is not known. Similar to current findings, sex differences following prenatal stress or prenatal alcohol exposure on adult HPA and depression-like behavioral measures have been described in a series of studies. Some studies imply these sexually dimorphic effects are due to the suppression of the late gestational phase fetal testosterone surge by the prenatal manipulation (Viau, et al, 1993; Ward and Weisz, 1980) resulting in diminished masculinization of the male offspring. While this is an attractive explanation considering that maternal adrenalectomy also diminishes the fetal testosterone surge (Sinha et al, 1997), it does not explain the profound effect of prenatal stress and fetal alcohol exposure on the female offspring, although it may cause the observed effect of maternal adrenalectomy on the male offspring's HPA function. The data that maternal adrenalectomy leads to increased ACTH – but normal CORT responses in male offspring, could be also explained by adrenal sensitivity to ACTH being affected in male offspring of ADX dams.

The lack of treatment effects in CORT stress response did not correlate with the altered hippocampal and/or hypothalamic GR expression observed in the offspring. Similarly to studies of prenatal stress or prenatal dexamethasone treatment, where alterations in hippocampal GR expression were found in both male and female offspring (Koehl et al., 1999; Brabham et al., 2000), we also found no significant sex differences in GR expression in response to maternal adrenalectomy; a finding that needs to be explored in the future.

Our behavioral measures suggested that maternal ADX increased depression-like behavior of adult offspring, particularly females. This observation is similar to those where animals prenatally stressed or exposed to elevated glucocorticoids show increased depression-like behavior (Weinstock, 2002; Drago, 1999), but there is no direct evidence to date that these behavioral deficits are the direct consequence of elevated maternal glucocorticoids (Weinstock, 2005). Both increased and decreased glucocorticoids lead to the same behavioral consequence, as seen in depressive behavior (Kademian, 2005), representing the mostly forgotten principal expressed in the Yerkes-Dodson law (Yerkes and Dodson, 1908). It predicts an inverted U-shaped function between arousal and performance. However, the idea that high and low maternal glucocorticoids can result in the same behavioral outcome in the adult offspring is a new finding and merits further investigation.

Since neither CORT substitution regimen appears to fully compensate for ADX-induced alterations in the offspring, we cannot exclude the idea that disappearance of a diurnal rhythm in maternal CORT levels affects fetal development. However, the present study has shown that decreased maternal glucocorticoids developmentally program the HPA axis and depression-like behavior in a sexually dimorphic manner. Further studies are necessary to determine the mechanism by which this prenatal programming occurs.

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Figure 1. Body weight in adult female and male offspring at 80–90 days of age. (n=11–23/sex/treatment). Treatment groups include sham-adrenalectomized (SHAM, n=5), adrenalectomized with placebo pellet (ADX, n=5), adrenalectomized with 50 mg corticosterone release pellet (ADX+CORT50, n=5) or adrenalectomized with 75 mg corticosterone release pellet (ADX+CORT75, n=5) dams. Values are shown as mean $\pm$ SEM. Asterisk indicates significant difference from SHAM group, $p < 0.05$, using post-hoc Tukey HSD.

Figure 2.

Plasma ACTH and CORT levels at 0 (basal), 30, 45 and 60 minutes after the onset of a 30-minute restraint stress in SHAM, ADX, ADX+CORT50, and ADX+CORT75 (a) females (n=6–8 animals/time point) and (b) males (n=7–8 animals/time point) at 80–90 days of age. One to two animals per litter were used for each time point. The experiment was carried out between 1000 and 1200h. Values are shown as mean $\pm$ SEM. Asterisk indicates significant difference from SHAM group at specific time point, $p < 0.05$, using post-hoc Tukey HSD. The basal ACTH and CORT values have been previously reported (Slone-Wilcoxon and Redei, 2004).

Figure 3.

Levels of GR mRNA in the hippocampus and hypothalamus of rats (70–80 days of age, $n=8-10$ /group). No sex differences were found in GR mRNA levels in any of the brain regions, so male and female data are pooled. (a) Representative blot is shown. Total RNA (8–10 μ g/lane) was analyzed. (b) Specific mRNA levels were normalized to the β -actin mRNA level of each sample. Values are means $\pm$ SEM. Asterisk indicates significant difference from SHAM control, $p<0.05$.

A.

B.

C.

Figure 4.

Time spent in inner circle (**A**) and total distance traveled (**B**) in the 10-minute open field test for SHAM, ADX, ADX+CORT50 and ADX+CORT75 pups at 21 days of age (1–2 pups/sex/litter). Immobility in the forced swim test (**C**) measured in 5 sec bins. Adult female and male SHAM, ADX, ADX+CORT50 and ADX+CORT75 offspring at 60–70 days of age (n=2–3 animals/sex/litter) were tested. Values are mean $\pm$ SEM. Asterisk indicates significant difference from SHAM control, $p < 0.05$.

Table 1

Maternal and neonatal plasma corticosterone levels on postnatal day 1.

Group	CORT (ng/ml)
<i>Mothers</i>	
SHAM	41.8 $\pm$ 5.8
ADX	8.50 $\pm$ 2.6*
ADX+CORT50	17.1 $\pm$ 6.2*
ADX+CORT75	68.9 $\pm$ 6.7*
<i>Neonates</i>	
SHAM	47.1 $\pm$ 10.4
ADX	89.7 $\pm$ 12.7*
ADX+CORT50	93.6 $\pm$ 14.8*
ADX+CORT75	41.1 $\pm$ 15.4

Samples are from all mothers (n=5/treatment) but only one male and one female pup from each litter (n=5/sex/treatment). There were no sex differences in the neonates, so males and females are combined. Values are shown as mean $\pm$ SEM. Asterisk indicates significant difference from SHAM group, $p < 0.05$. We have previously reported these data (Slone-Wilcoxon and Redei, 2004).