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Effects of early life social stress on endocrinology, maternal behavior, and lactation in rats

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Abstract

Exposure to early life stress is a predictor of mental health disorders, and two common forms of early life stress are social conflict and impaired maternal care, which are predominant features of postpartum mood disorders. Exposure of lactating female rats to a novel male intruder involves robust social conflict and induces deficits in maternal care towards the F1 offspring. This exposure is an early life social stressor for female F1 pups that induces inefficient lactation associated with central changes in oxytocin (OXT), prolactin (PRL), and arginine vasopressin (AVP) gene expression in adult F1 females.

The mothers of the rats in the current study were either allowed to raise their pups without exposure to a social stressor (control), or presented with a novel male intruder for 1 hour each day on lactation days 2-16 (chronic social stress). The effects of this early life chronic social stress (CSS) exposure on subsequent peripheral endocrinology, maternal behavior, and physiology were assessed.

Exposure of female pups to early life CSS resulted in persistent alterations in maternal endocrinology at the end of lactation (attenuated prolactin and elevated corticosterone), depressed maternal care and aggression, increased restlessness and anxiety-related behavior, impaired lactation, and decreased saccharin preference. The endocrine and behavioral data indicate that early life CSS has long-term effects which are similar to changes seen in clinical populations of depressed mothers, and provide support for the use of the chronic social stress paradigm as an ethologically relevant rodent model for maternal disorders such as postpartum depression and anxiety.

Keywords

Stress; early life; postpartum depression; anxiety; lactation; estradiol; prolactin; corticosterone

INTRODUCTION

Exposure to early life stress is a potent predictor of mental health disorders in adulthood (Bale et al., 2010; Heim and Binder, 2011; Lupien et al., 2009), and one common form of early life stress is impaired maternal care, which is a predominant feature of postpartum

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mood disorders (Paulson et al., 2006). Both natural variations in maternal care and maternal neglect can have profound and enduring effects on offspring behavioral endocrinology, particularly the hypothalamic pituitary adrenal (HPA) axis (Meaney and Szyf, 2005). Many of the behavioral and physiological effects of early life stressors on offspring are directly mediated by changes in maternal care (Meaney and Szyf, 2005; Weaver et al., 2004).

The exposure of lactating rat dams to chronic social stress (CSS) is an effective and ethologically relevant model of postpartum depression (Nephew and Bridges, 2011). Furthermore, CSS during lactation induces deficits in maternal care towards the F1 offspring, and studies indicate that this early life CSS decreases nursing efficiency and maternal aggression during lactation in the adult female offspring of CSS exposed dams. These effects on maternal behavior are associated with attenuated oxytocin (OXT), prolactin (PRL), and arginine vasopressin (AVP) gene expression in the hypothalamus and amygdala (Murgatroyd and Nephew, 2013). The current study augments this recent investigation of early life CSS associated changes in central gene expression through the analysis of plasma hormones (OXT, estradiol, PRL, and corticosterone), and the inclusion of milk intake to assess lactation and saccharin preference as an abstract measure of anhedonia.

Changes in female gonadal hormones are involved in the etiology of mood disorders in women (Rubinow, 2006), and it has been postulated that exogenous estradiol is a valid target for the treatment of postpartum mood disorders through its effects on normalizing estradiol sensitive maternal neural circuits (Bloch et al., 2000; Moses-Kolko et al., 2009). Estradiol is involved in the expression of both OXT and AVP receptors in the vertebrate brain (Bale and Dorsa, 1995; Champagne et al., 2001; Ochedalski et al., 2007), and has also been linked to maternal depression (Dennis et al., 2008; Gregoire et al., 1996; Moses-Kolko et al., 2009; Studd, 2011). Estrogen treatment increases OXT mRNA levels in the ventromedial hypothalamus of ovariectomized female rats (Bale and Dorsa, 1995; Bale et al., 1995), and in women with elevated plasma estradiol, OXT levels are also higher (Amico et al., 1981). It was hypothesized that the effects of early life CSS on OXT expression in dams is mediated by long-term changes in estradiol which are established during developmental exposure to early life CSS.

Oxytocin is an important mediator of several social behaviors, including maternal care (Champagne et al., 2001; Pedersen and Prange Jr., 1979) and maternal aggression (Bosch et al., 2004; Bosch et al., 2005; Giovenardi et al., 1997; Nephew et al., 2009). Low plasma OXT is associated with an increased risk for postpartum depression (Skrundz et al., 2011) and depressed mothers display changes in the neural response to exogenous OXT (Strathearn et al., 2009). OXT is also a key physiological mediator of lactation in terms of milk let-down (Grosvenor et al., 1986; Parker et al., 1991; Uvnäs-Moberg and Eriksson, 1996), and the previous neuroendocrine findings suggest that peripheral levels may be attenuated in offspring exposed to early life CSS (Murgatroyd and Nephew, 2013). It was hypothesized that early life CSS will induce decreases in basal plasma OXT that will be associated with depressed maternal care and/or deficiencies in lactation.

The brain prolactin system mediates the stress response during pregnancy. Activation of the central PRL system inhibits HPA activity during pregnancy, allowing for typical maternal responses (Torner and Neumann, 2002). PRL has also been implicated in both the behavioral and physiological changes of pregnancy and lactation that are necessary for successful offspring care (Bridges et al., 1990; Grattan, 2002), and low plasma levels of PRL are associated with postpartum depression (Abou-Saleh et al., 1998). Our previous report of inefficient lactation and decreased PRL receptor expression in the hypothalamus indicated that PRL may be involved in both the behavioral and physiological effects of early

life CSS. It was hypothesized that deficiencies in nursing behavior and/or milk intake by pups will be associated with decreased basal PRL levels.

Several types of stressor exposures can lead to the disruption of HPA axis activity, and this disruption often mediates symptoms of depression (Hammen, 2005). Exposure to maternal separation or low maternal care induces alterations in the maternal neural circuitry required for appropriate maternal responses and increases in the expression of behaviors associated with depression and anxiety (Murgatroyd et al., 2009; Murgatroyd and Spengler, 2011; Murgatroyd et al., 2004). Plasma corticosterone was assessed in the present study to measure the effects of early life CSS on the HPA axis of lactating dams, a potential mediator of the behavioral and physiological effects of the treatment. It was hypothesized that early life CSS will elevate basal plasma corticosterone levels.

In summary, it was postulated that early life CSS exposed females will display behavioral and physiological changes often reported in depressed mothers, including decreased basal plasma prolactin, estradiol, and oxytocin and elevated basal corticosterone, depressed maternal behavior, and deficiencies in lactation.

MATERIALS AND METHODS

Animals

Animals in this study were maintained in accordance with the guidelines of the Committee of the Care and Use of Laboratory Animals Resources, National Research Council, and the research protocol was approved by the Tufts Institutional Animal Care and Use Committee. “CSS dams” refers to the adult females exposed to chronic social stress during lactation (F0), and “early life CSS dams” refers to the adult female offspring of the CSS dams; the focus of the present study (F1).

CSS Model: Creation of F0 Dams

The CSS dams (Charles River, Wilmington, MA) were subjected to a chronic social stress protocol from days 2 to 16 of lactation as reported in (Nephew and Bridges, 2011). This procedure consisted of placing a similarly sized (220–300 g) novel male intruder into a lactating female's home cage for one hour from day 2 to 16 of lactation. Control dams were not exposed to the CSS protocol; they were only tested for maternal care and maternal aggression on days 2, 9, and 16 of lactation. The pups are left in the cage during the intruder presentation, and the CSS exposure results in depressed maternal care and increased anxiety-related behavior and maternal aggression (Nephew and Bridges, 2011).

Early life CSS: Creation of F1 Females

The control and early life CSS F1 females of the current study were the offspring of the F0 control and CSS dams; the differences between the treatments of the control and early life CSS F1 females were limited to the exposure of the early life CSS F1 females to depressed maternal care and conflict between their F0 mothers and the male intruders during age 2 to 16 days. The F1 control and early life CSS animals were treated identically after the age of 16 days. After weaning all F1 pups on day 23, the female offspring from the twelve control and twelve CSS dams were housed in groups of four until 70 days of age when two from each litter were mated with 6 proven breeder males (24 F1 females for the control and early life CSS groups). Successful mating was determined by body weight gain. One juvenile and one adult control and early life CSS female from each litter were randomly selected on day 35 or 60 and euthanized between 0800 and 1000 for trunk blood collection used on endocrine analysis (n's=12). Estrous cycle was not assessed at any point to reduce manipulation of the animals. Total F2 pup number and litter weights were recorded on the

day of parturition, and litters were then culled to four females and four males. The final sample sizes were 12 dams for the control group and 14 dams for the early life CSS group.

Maternal Behavioral testing

Maternal care and maternal aggression were assessed on days 2, 9, and 16 of lactation (early, mid, and late lactation) between 09:00 and 12:00h in all dams to assess the effects of early life CSS at different time points during lactation as previously reported (Murgatroyd and Nephew, 2013). After a 60-min pup removal, maternal care testing consisted of the re-introduction of all eight pups to the home cage video recording the dam for 30 minutes. These 30 min behavior observations produce consistent and substantial behavioral data that are similar to observations of undisturbed maternal care over 30 and 60 min (Byrnes et al., 2000; Johnson et al., 2011). The latencies to retrieve pups back to the nest and initiate nursing, and frequencies and durations of pup retrieval, pup grooming, nursing, nesting, self-grooming, and general locomotor activity were scored by an observer who was blind to the treatment using ODLog behavioral analysis software (Macropod Inc., USA). Nursing behavior scoring was started when the dam had been motionless over the litter for longer than 10 seconds, and stopped whenever she moved off the pups. Nursing latency is defined as the period of time from the start of the maternal care observation to the initial expression of nursing behavior, and nursing duration is the cumulative nursing behavior during the 30 min maternal care observation. Mean nursing bout for each animal was calculated by dividing nursing duration by nursing frequency. Nesting was defined as manipulation of the nesting material with mouth or paws. Total maternal care included the combined durations of pup grooming and nursing. The composite measure of non-maternal behavior included the durations of self-grooming, nesting, and locomotor activity. Following the 30 minute maternal care video recording, the pups were left undisturbed for another 90 minutes (without video recording) to provide a 120 minute nursing period to assess milk intake through the weighing the litter. Milk intake was calculated by subtracting the litter weight at the start of the maternal care observation from the litter weight 120 minutes later.

Following the maternal care testing and nursing period, the pups were weighed, returned to the cage, and a similarly sized (225–300 g) novel virgin male Sprague Dawley intruder was introduced for a 30 minute maternal aggression test. Dams were never exposed to the same male twice. The latency to initiate aggression and the frequency and durations of attacking (boxing or tackling), biting, kicking, pinning to the bottom of the cage, self-grooming, and locomotor activity were scored. Total aggression included the durations of attacks, bites, kicks, and pins.

Saccharin Preference Testing

Pre-weighed bottles of water and saccharin were placed in the dams cages at 1600 on lactation days 2, 9, and 16 (alternating left or right sides of cage). Bottles were removed and weighed the next morning at 0800 to determine the % of saccharin intake out of total fluid intake over the 16 hour period.

Endocrine Assays

Plasma corticosterone and estradiol were measured by commercial RIA kits (Siemens), prolactin was measured using the RIA kit from Dr. A.F. Parlow at the National Hormone and Peptide Program, Harbor-UCLA Medical Center, and oxytocin was measured by commercial ELISA (Enzo Life Sciences). All samples were run in duplicate. Assay sensitivities were as follows: corticosterone = 5.7ng/ml, prolactin = 0.5ng/ml, estradiol = 8pg/ml, oxytocin = 11.7pg/ml. Intraassay variability was 3-7%, and interassay variability between assays in the Nephew lab is 5-10%.

Statistics

The effects of early life CSS on mean pup bodyweight across lactation, behaviors, milk intake, and saccharin preference were tested using 2-way repeated measures ANOVAs with lactation days 2, 9, and 16 as the repeated factor. Bonferroni adjustments were used to make pairwise comparisons on individual lactation days when there were significant interactions between treatment and lactation day. The effects of early life CSS treatment on hormone levels were compared with 1-way ANOVA's at each age due to the numerous age-related differences between the groups. All graphical results are presented as mean + SEM, the level of statistical significance was $p < 0.05$, and effect sizes were presented for all differences (η^2 for 1-way ANOVA, and η^2 for 2-way ANOVA).

RESULTS

Pup Growth

There were no group differences in the dam weights or numbers or weights of pups at birth, or across lactation (days 2, 9, or 16, all p 's > 0.05).

Basal Plasma Estradiol, Prolactin, Corticosterone, and Oxytocin

Basal estradiol concentrations were elevated in adult early life CSS females ($F_{1,25}=8.4$, $p<0.01$, $\eta^2=0.20$), compared to age matched controls (fig. 1). Basal PRL concentrations were lower in early life CSS dams ($F_{1,25}=14.7$, $p<0.01$, $\eta^2=0.27$ fig. 2). Basal corticosterone concentrations in adult ($F_{1,25}=4.6$, $p=0.04$, $\eta^2=0.17$) and lactating ($F_{1,25}=10.7$, $p<0.01$, $\eta^2=0.32$) early life CSS females were elevated compared to controls (fig. 3). Oxytocin levels in juvenile females (control = 258.9 ± 45.7 , early life CSS = 341.4 ± 52.3 pg/ml) adult females (control = 286.4 ± 34.7 , early life CSS = 343.6 ± 55.1 pg/ml) and on lactation d23 (control = 896.3 ± 73.5 , early life CSS = 1005.0 ± 89.6 pg/ml) were similar (n 's = 12-14, all p 's > 0.2).

Maternal Care

The duration ($F_{1,25}=11.4$, $p<0.01$, $\eta^2=0.09$ fig. 4) and frequency (data not presented, $F_{1,25}=10.7$, $p<0.01$, $\eta^2=0.09$) of pup retrieval was elevated in early life CSS dams during lactation and there were significant effects of time on both retrieval duration ($F_{1,25}=22.8$, $p<0.01$, $\eta^2=0.34$ fig. 4) and frequency (data not presented, $F_{1,25}=25.9$, $p<0.01$, $\eta^2=0.09$). Since the number of pups was controlled between treatment groups throughout the study, this increase represents less efficient retrieval by the stress group, often involving atypical moving of the nest site and/or walking around the cage while carrying a pup for extended periods. There were effects of treatment ($F_{1,25}=5.6$, $p<0.05$, $\eta^2=0.06$) and time ($F_{2,52}=8.6$, $p<0.01$, $\eta^2=0.14$) on the duration of pup grooming during lactation, where early life CSS decreased pup grooming (fig. 5), and there was also an increase in nursing latency in the stressed dams (treatment: $F_{1,25}=5.6$, $p<0.05$, $\eta^2=0.13$, time: $F_{2,52}=5.5$, $p<0.05$, $\eta^2=0.07$ fig. 6). When the dams did settle down to nurse, there were significant effects of early life CSS and time on nursing duration (treat: $F_{1,25}=7.9$, $p<0.05$, $\eta^2=0.08$, time: $F_{2,52}=29.7$, $p<0.01$, $\eta^2=0.36$ fig. 7) with an interaction between time and treatment on day 2 ($F_{2,48}=3.4$, $p<0.05$, $\eta^2=0.04$ fig. 7). These effects on pup grooming and nursing contributed to an overall depression of total maternal care (treat: $F_{1,25}=14.4$, $p<0.01$, $\eta^2=0.14$, time: $F_{2,52}=54.8$, $p<0.01$, $\eta^2=0.040$) with a significant interaction on day 2 ($F_{2,48}=9.3$, $p<0.01$, $\eta^2=0.07$ fig. 8). The early life CSS dams took longer to settle down on the nest and spent less time grooming and nursing their litters. The decrease in total maternal care duration was largely driven by the 79% decrease in nursing, which was associated with an attenuation in mean nursing bout duration in the early life CSS dams on day 2 (control = 69.3 ± 11.9 , early life CSS = 20.3 ± 3.0 s, time: $F_{2,52}=7.14$, $p<0.15$, $\eta^2=0.04$, interaction: $F_{2,48}=9.3$, $p<0.01$,

$\eta^2=0.07$). Instead of displaying direct maternal care, the early life CSS dams spent more time on non-maternal behaviors, with a significant increase on day 2 (treatment: $F_{1,25}=11.6$, $p<0.01$, $\eta^2=0.08$, time: $F_{2,52}=52.7$, $p<0.01$, $\eta^2=0.50$, interaction: $F_{2,48}=5.9$, $p<0.01$, $\eta^2=0.06$ fig. 9). In summary, the early life CSS dams exhibited depressed maternal care and an increase in self grooming, nesting and locomotion.

Milk Intake by Pups

Milk intake per litter by early life CSS pups was significantly lower following the 120 minute nursing period compared to controls (treatment: $F_{1,25}=4.1$, $p<0.05$, $\eta^2=0.04$, time: $F_{2,52}=15.7$, $p<0.01$, $\eta^2=0.31$ fig. 10).

Maternal Aggression

The early life CSS dams spent less time attacking on day 2 of lactation (treatment: $F_{1,25}=7.1$, $p<0.01$, $\eta^2=0.12$, time: $F_{2,52}=4.8$, $p<0.05$, $\eta^2=0.09$, interaction: $F_{2,48}=3.5$, $p<0.05$, $\eta^2=0.06$ fig. 11). There was also an overall decrease in biting in the early life stressed F1 dams (treatment: $F_{1,25}=6.1$, $p<0.05$, $\eta^2=0.07$, fig. 12) which contributed to the overall decrease in maternal aggression (treatment: $F_{1,25}=7.5$, $p<0.05$, $\eta^2=0.11$, time: $F_{2,52}=3.8$, $p<0.05$, $\eta^2=0.09$, fig. 13). During the aggression tests, the early life stressed dams spent more time self grooming (control = $56.9 \pm 8.4s$, stress = $36 \pm 14.9s$ treatment: $F_{1,25}=4.0$, $p=0.05$, $\eta^2=0.06$, time: $F_{2,52}=3.8$, $p<0.05$, $\eta^2=0.13$)

Saccharin Preference

Saccharin preferences was decreased in the early life stress dams during lactation compared to controls (treatment: $F_{1,25}=7.8$, $p<0.05$, $\eta^2=0.08$, fig. 14).

DISCUSSION

Exposure of female pups to early life CSS, which includes both depressed maternal care and maternal aggression during development, resulted in persistent alterations in maternal endocrinology, depressed maternal care and aggression, restlessness, impaired lactation, and decreased saccharin preference. The endocrine data indicate that early life CSS has long-term effects on basal estradiol, PRL, and corticosterone which are similar to changes seen in clinical populations of depressed mothers. The early life CSS paradigm is ethologically relevant and induces several behavioral changes often specifically associated with postpartum mood disorders, such as depressed maternal care and restlessness. It is concluded that early life CSS in rodents is a useful model for several of the endocrine, physiological, and behavioral features of postpartum mood disorders.

The overall changes in estradiol, PRL, and corticosterone throughout development are similar to observations in humans. However, the endocrine sampling was only at the end of lactation, where the behavioral data were collected throughout lactation. Estradiol has been successfully used as a treatment option for depression in mothers (Moses-Kolko et al., 2009). The lack of differences in juvenile estradiol levels in the present study indicates that the effect of early life CSS on plasma estradiol may be mediated by pubertal changes in endocrine physiology. Although estradiol values during late pregnancy are not available, the adult values indicate that peripheral estradiol regulation is altered by exposure to early life CSS, resulting in elevated estradiol levels in the adult early life CSS females. It has been hypothesized that the sudden decrease in gonadal hormones from late pregnancy to lactation may be involved in the etiology of postpartum depression (Bloch et al., 2003), as the simulation of elevated estrogen and progesterone levels followed by withdrawal induces depressive symptoms in women with a history of depression (Bloch et al., 2000). While we do not have early postpartum samples, it is possible that early life CSS dams experienced a

more severe decrease in estrogen during early lactation. This hypothesis of a more robust decrease and/or lower estradiol levels during early lactation is supported the previously reported decreases in central OXT and OXT receptor expression (Murgatroyd and Nephew, 2013), as estrogen mediates central OXT activity (Bale et al., 1995; Champagne et al., 2001). The early life CSS model can be a useful tool to test the effects of sex steroid manipulations on postpartum mood disorders symptoms.

In addition to the lower levels of central PRL receptors previously reported (Murgatroyd and Nephew, 2013), peripheral PRL is also attenuated in dams exposed to early life CSS. PRL is an important component of both maternal care and lactational physiology, and the suppressed peripheral basal PRL levels in the early life CSS dams are associated with depressed maternal care and impaired lactation. It is possible that the lack of effects in the juveniles and adult females were due to pubertal changes and variation in estrous stage. The decreased levels of PRL and elevated corticosterone in dams support the hypothesis presented by Torner and Neumann that PRL mediates the suppression of the HPA axis during lactation (Torner and Neumann, 2002). As with estrogen, low plasma levels of PRL are associated with maternal depression, supporting research on PRL as a treatment target for treating stress related disorders that affect both maternal care and lactation, such as postpartum depression and anxiety (Stuebe et al., 2012).

The corticosterone data support the wealth of literature on the role of the HPA axis in mood disorders (Pariante and Lightman, 2008) and parallel recent reports in clinical studies of early life stress, where exposure to both maternal depression and family anger are associated with elevated basal corticosterone (Essex et al., 2011). Elevated levels of corticosterone are associated with anxiety and disruptions in maternal care, and the current study reports impaired maternal care that includes both depressed maternal care and increases in non-maternal behaviors which indicate restlessness/anxiety. Although the difference is not significant at the juvenile stage, the corticosterone elevations in early life CSS adult females and dams indicates that this stress paradigm has persistent effects on HPA activity. As with estradiol and PRL, the lack of a difference at the juvenile stage may indicate that the changes in nulliparous adults and dams may be mediated by pubertal development, and/or that individual variation in the impact of early life CSS is higher in juveniles. It is likely that adult depression and anxiety-like behavior is increased in the adult females as well, but this requires further investigation. It is postulated that the increase in HPA activity, in conjunction with the decreases in estradiol and prolactin, mediates the decrease in maternal care and increases in nesting and locomotor activity during maternal care testing, making it less likely that the early life CSS exposed dams quickly gather their pups and settle down for sustained periods of pup grooming and nursing. As both acute and chronic stress are inhibitory towards lactation (Lau, 2001), the elevation in corticosterone may mediate the impaired milk intake by the offspring of early life CSS dams.

In general, exposure to early life CSS had similar effects on maternal care as CSS during lactation; pup directed behaviors were depressed, and non-pup directed behaviors indicative of anxiety increased. The differences in maternal care effects between this study and our previous investigation of early life CSS (Murgatroyd and Nephew, 2013) are postulated to be due to the recording of compensatory behavior in the initial report resulting from higher levels of ambient activity in the animal facility prior to the behavioral observations. It should be noted that both studies conclude that early life CSS adversely affects subsequent maternal care. Considering the absence of additional stressors during lactation, the attenuation of maternal care on lactation day 2 in the early life stressed dams is robust, especially compared to the pup care of dams stressed during lactation (Nephew and Bridges, 2011). Early life CSS exposure affected multiple components of typical maternal responding, starting with the retarded gathering of the pups to the nest for grooming and nursing. When

the stressed dams did settle down to groom and nurse, the durations were short and often interrupted by nesting, locomotor activity, or simply time off the nest. Taken together, the differences in nursing latency and durations of nursing, and the 30 minute observation period suggest that the effects on maternal care were not solely a result of an initial delay to respond to pups; the potential nursing time of early life CSS dams was not severely limited by the increased nursing latencies. When compared to major depression in females, postpartum depression is more likely to be associated with restlessness/agitation and anxiety (Bernstein et al., 2008; Jolley and Betrus, 2007), and it is postulated that the increase in nesting, self-grooming, and activity in the context of depressed maternal care is indicative of ethologically relevant increases in restlessness. The most robust effects of early life CSS on both maternal care and non-maternal behaviors were on day 2, with significant treatment x lactation day interactions on days 9 or 16. This pattern suggests that the early life dams were modulating their maternal responsiveness across lactation, possibly attempting to compensate for early deficiencies in maternal care in response to vocalizations from the pups (Okabe et al., 2013).

Depressed milk intake during lactation was associated with a substantial decrease in nursing behavior on day 2, supporting the hypothesis that the pups of early life CSS dams obtained very little milk at this time point through a decrease in maternal care. It is suggested that the lack of significant differences between the groups on days 9 and 16 represent attempts by the early life CSS dams to compensate for the deficient nursing early in lactation, possibly in response to cues from the pups (Okabe et al., 2013). The ability to continue to compensate during mid and late lactation, when milk needs are higher due to pup growth, may be impaired by attenuations in central OXT (Murgatroyd and Nephew, 2013). While overall compensation was successful in terms of pup body weight, but this was in the presence of continuous access to food and water and a benign maternal environment with few stressors. It is unknown if there were transient or less obvious effects on pup growth during days 2-9 of lactation, or if a more challenging environment where resources were limited and/or included more significant stressors would have affected pup growth. The current growth data are similar to work in humans, where major effects of postpartum depression are most likely to be observed in populations subjected to high levels of stress and limited access to food, such as low socioeconomic groups (Adewuya et al., 2008; Stewart, 2007). Overall, the nursing and milk intake data parallel clinical studies of lactation in depressed mothers, where depression is associated with nursing difficulties and early cessation (Stuebe et al., 2012).

The maternal aggression data directly support our recent report of attenuated aggression in early life CSS exposed dams (Murgatroyd and Nephew, 2013), which contrasts with the effects of CSS on dams (Nephew and Bridges, 2011). This contrast is not surprising given the lack of intruder exposure during lactation in early life CSS dams and the hypothesis that the increased aggression in CSS dams is due to sensitization to the intruder exposure. The increase in self-grooming during aggression testing on day 16 indicates that CSS dams were more anxious in a challenging behavioral context (Denmark et al., 2010), supporting our previous work (Murgatroyd and Nephew, 2013). One potential mediator of these effects on aggression and anxiety-like behavior is the elevation in basal corticosterone, as it has been postulated that the stress hyporesponsiveness of lactating dams mediates the decrease in fear and anxiety that is associated with the display of maternal aggression (Lonstein and Gammie, 2002). Another potential neural mediator is OXT (Bosch et al., 2005; Bosch and Neumann, 2012). While there was no effect of early life CSS on basal OXT in the current study, levels were only assessed on day 23, so it is possible that basal oxytocin levels were different during early lactation when aggression was attenuated. Early life CSS does have long term suppressive effects on central OXT and OXT receptor expression, and this attenuated OXT activity may mediate the lower levels of aggression during lactation

(Murgatroyd and Nephew, 2013). Although it might be expected to also see specific differences in aggressive behaviors at other points of lactation besides day 2, the expression of maternal aggression may not be as dependent on OXT in the hypothalamus or amygdala during mid and late lactation. A similar changing role of a nucleus across lactation has been described by Pereira and Morrell (Pereira and Morrell, 2009).

The decrease in saccharin preference on during lactation supports the hypothesis that the effect of early life CSS on milk intake is mediated by an anhedonia-associated behavioral mechanism during the initial response to pups. However, as noted previously, the nursing duration data on days 9 and 16 indicate that the stressed dams do compensate for the delay in maternal response. It is argued that measures of maternal care, the naturally occurring reward mediated behavior that is a primary treatment target, are the more sensitive, informative, and translationally relevant measures of anhedonia in animal studies of postpartum mood disorders.

In conclusion, early life CSS exposure has long term effects on the basal maternal endocrinology, depresses maternal care and aggression, increases restlessness and anxiety-related behavior, and impairs lactation. The decrease in basal prolactin, increase in basal corticosterone, depressed maternal care and saccharin preference, and impaired lactation in the current study of early life CSS exposed dams add to the previous report of decreased nursing efficiency and associated decreases in central OXT, PRL, and vasopressin expression (Murgatroyd and Nephew, 2013). Taken together with the initial study on the CSS model (Nephew and Bridges, 2011), exposure to chronic social stress impairs the maternal care of both maternal rats and their female offspring. The CSS model has many parallels with postpartum depression and anxiety in terms of both etiology and endocrine, physiological, and behavioral effects which make it a valuable tool for the study of novel preventative measures and treatments and for these disorders and their transgenerational effects.

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Highlights

- Early life chronic social stress is an ethologically relevant stress paradigm.
- Exposure to early life chronic social stress disrupts female endocrinology.
- Endocrine changes are associated with depressed maternal care and anxiety.
- Early life chronic social stress can be used to model postpartum depression.

Figure 1. Basal plasma estradiol concentrations were elevated in early life CSS adult females compared to levels in age matched controls. * indicates significant effect of early life CSS treatment, $p < 0.05$.

Figure 2. Basal plasma prolactin levels were decreased in dams on day 23 of lactation compared to levels in age matched controls. * indicates significant effect of early life CSS treatment, $p < 0.05$.

Figure 3. Basal plasma corticosterone concentrations were elevated in early life CSS female adults and dams on day 23 of lactation. * indicates significant effect of early life CSS treatment, $p < 0.05$.

Figure 4.

The mean duration of pup retrieval was increased in early life CSS dams compared to control dams. * indicates significant overall effect of early life CSS treatment, # indicates a significant overall effect of lactation day, $p < 0.05$.

Figure 5.

The mean duration of pup grooming was decreased in early life CSS dams compared to control dams during lactation. * indicates significant overall effect of early life CSS treatment, # indicates a significant overall effect of lactation day, $p < 0.05$.

Figure 6.

Mean nursing latency was longer in early life CSS dams compared to control dams. * indicates significant overall effect of early life CSS treatment, # indicates a significant overall effect of lactation day, $p < 0.05$.

Figure 7.

Mean nursing duration was shorter on day 2 in the early life CSS dams compared to control dams. * indicates significant overall effect of early life CSS treatment, # indicates a significant overall effect of lactation day, ** indicates a significant interaction between treatment and lactation day on day 2, $p < 0.05$.

Figure 8.

Mean maternal care was shorter on day 2 in the early life CSS dams compared to control dams. * indicates a significant overall effect of early life CSS treatment, # indicates a significant overall effect of lactation day, ** indicates a significant interaction between treatment and lactation day on day 2, $p < 0.05$.

Figure 9.

Mean non-maternal behavior was elevated on day 2 in the early life CSS dams compared to control dams. * indicates a significant overall effect of early life CSS treatment, # indicates a significant overall effect of lactation day, ** indicates a significant interaction between treatment and lactation day on day 2, $p < 0.05$.

Figure 10.

Mean milk intake by the pups of early life CSS dams was decreased compared to control dams. * indicates a significant overall effect of early life CSS treatment, # indicates a significant overall effect of lactation day, $p < 0.05$.

Figure 11.

Mean duration of attacking was decreased on lactation day 2 in early life CSS dams compared to control dams. * indicates a significant overall effect of early life CSS treatment, # indicates a significant overall effect of lactation day, ** indicates a significant interaction between treatment and lactation day on day 2, $p < 0.05$.

Figure 12. Mean duration of biting was decreased in early life CSS dams compared to control dams. * indicates a significant overall effect of early life CSS treatment, $p < 0.05$.

Figure 13.

Mean duration of maternal aggression was decreased in early life CSS dams compared to control dams. * indicates a significant overall effect of early life CSS treatment, # indicates a significant overall effect of lactation day, $p < 0.05$.

Figure 14.

Mean percentage saccharine intake was decreased in early life CSS dams compared to control dams. * indicates a significant overall effect of early life CSS treatment, $p < 0.05$.