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Gestational Cortisol and Social Play Shape Development of Marmosets' HPA Functioning and Behavioral Responses to Stressors

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Abstract

Both gestational cortisol exposure (GCE) and variability in postnatal environments can shape the later-life behavioral and endocrine outcomes of the hypothalamic-pituitary-adrenal (HPA) axis. We examined the influence of GCE and social play on HPA functioning in developing marmosets. Maternal urinary cortisol samples were collected across pregnancy to determine GCE for 28 marmoset offspring (19 litters). We administered a social separation stressor to offspring at 6, 12, and 18 months of age, during which we collected urinary cortisol samples and behavioral observations. Increased GCE was associated with increased basal cortisol levels and cortisol reactivity, but the strength of this relationship decreased across age. Increased social play was associated with decreased basal cortisol levels and a marginally greater reduction in cortisol reactivity as offspring aged, regardless of offspring GCE. Thus, GCE is associated with HPA functioning, but socially enriching postnatal environments can alter the effects associated with increased fetal exposure to glucocorticoids.

Keywords

fetal programming; social play; HPA axis; prenatal cortisol; cortisol reactivity; development; non-human primate; marmoset

INTRODUCTION

There is a robust link between glucocorticoid exposure during pregnancy and later-life outcomes in offspring including alterations in hypothalamic-pituitary-adrenal (HPA) axis activity, propensities for particular behavioral phenotypes, and vulnerabilities for adult diseases (Barker, 1990, 1998; Davis et al., 2007; Weinstock, 2008). An emerging model proposes that prenatal exposure to glucocorticoids (cortisol in primates; corticosterone in rodents) programs the fetus to adapt to the subsequent environmental demands, which may

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be signaled through changes in maternal physiology and behavior including the mother's own activation of glucocorticoids in response to stressful stimuli (Harris & Seckl, 2011; Glover, O'Connor, & O'Donnell, 2010). The prenatal period is a time where offspring undergo massive ontogenetic changes, particularly brain development, and, consequently, offspring phenotypes are exceptionally vulnerable to changes in the maternal uterine environment (Charil, Laplante, Vaillancourt, & King, 2010). Cortisol concentrations naturally rise during pregnancy in both nonhuman primates and humans (Mustoe, Birnie, Korgan, Santo, & French, 2012; Sandman et al., 2006), and alterations in metabolism, efficacy of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), or increases in maternal stress, anxiety, or depression can affect the fetus' exposure to glucocorticoids and later-life outcomes (Field, Diego, & Hernandez-Reif, 2006; Holmes et al., 2006). Given that glucocorticoids vary considerably among mothers during gestation, it appears that the effects of prenatal stress and other alterations in glucocorticoid exposure during pregnancy may alert fetuses to demands in their imminent environment (Glover, 2011). Importantly, the relationship between glucocorticoid exposure and later-life outcomes in offspring are evident when measuring maternal glucocorticoid levels directly via maternal plasma or amniotic fluid (Davis, Glynn, Waffarn, & Sandman, 2011; O'Connor, Bergman, Sarkar, & Sandman, 2012), or when using maternal stress or anxiety as proxy measures of glucocorticoids (Davis et al., 2007; O'Connor et al., 2005). Furthermore, early postnatal experiences, such as parental care and early-life adversity, can also play a major role in shaping developmental outcomes including HPA responsiveness (Birnie, Taylor, Cavanaugh, & French, 2013; Francis & Meaney, 1999; Laurent et al., 2013; Liu et al., 1997). Thus, the interaction between intra-uterine environments such as variation in glucocorticoid exposure and diverse postnatal experiences can mold the developmental trajectory of many endocrine and behavioral later-life outcomes.

Exposure to glucocorticoids can exert a long-lasting influence on normal HPA activity and the HPA response to stress. Adult male guinea pigs exposed to greater prenatal stress had a reduced basal and stress-induced plasma glucocorticoid concentrations, while females exhibited increased basal and stress-induced plasma glucocorticoid concentrations (Lingas & Matthews, 2001). In general, rats exposed to increased prenatal glucocorticoid exposure show HPA hyperactivity, that is, increase in circulating glucocorticoids, in response to stress (Barbazanges, Piazza, Le Moal, & Maccari, 1996; Weinstock, Poltyrev, Schorer-Apelbaum, Men, & McCarty, 1998); however, there are exceptions (Cannizzaro et al., 2006; Van den Hove et al., 2005). The HPA response to stress in rats appears to be sex specific, with females being more susceptible to the effects of prenatal programming on later-life HPA activity (Bhatnagar, Lee, & Vining, 2005; McCormick, Smyth, Sharma, & Meaney, 1995; Weinstock, 2007). Moreover, the effect of prenatal programming of later-life HPA activity is strongly linked to the timing of gestational stress, where the extent of perturbation of later-life HPA activity is often greatest when maternal stressors are imposed during the period of maximal fetal brain growth (Kapoor & Matthews, 2005). In primates, prenatal stress is associated with increased levels of ACTH and cortisol to both basal and stressed conditions (Clarke, Wittwer, Abbott, & Schneider, 1994) and increased basal cortisol levels following a dexamethasone (DEX) suppression in juvenile rhesus monkeys (Coe et al., 2003). Conversely, marmosets do not show an increase in basal cortisol following prenatal exposure

to DEX (Hauser et al., 2008). Studies on humans have shown a similar programming-like effect of prenatal cortisol exposure on HPA activity. Increased maternal cortisol levels or maternal reports of high stress or anxiety during pregnancy were positively associated with levels of cortisol across the day in children at 4–10 years of age (Gutteling, de Weerth, & Buitelaar, 2005; O'Connor et al., 2005) and this association between maternal anxiety and children's cortisol persists into adolescence (Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008). Unlike with fetal programming in rodents, the effect of prenatal cortisol exposure on HPA activity in primates does not appear to be as strongly linked to the timing of stressful stimuli during gestation (or the data are mixed). However, in cases where fetal programming is linked to gestational timing in primates, early- or mid-gestation exposure appears to be the more sensitive period (Mustoe et al., 2012; Schneider, Roughton, Koehler, & Lubach, 1999).

The programming effects of glucocorticoids are not limited to HPA activity; behavioral and cognitive programming appears to occur as well. Offspring exposed to higher levels of gestational glucocorticoids generally show more reactive temperaments and slower cognitive and motor development. For example, juvenile rhesus monkeys exposed to higher gestational cortisol show decreased levels of exploratory behavior and attention, while exhibiting increases in emotionality, inactivity, and immature motor reflexes and motor impairments (Clarke & Schneider, 1993; Clarke, Soto, Bergholz, & Schneider, 1996; Coe et al., 2003; Schneider, 1992; Schneider et al., 1999). Marmosets show similar responses to artificially-elevated gestational cortisol exposure (DEX treatment), in that offspring born to DEX-treated mothers show more inactivity and a decrease in initiating social play with siblings and parents compared to controls (Hauser et al., 2008). Human infants and toddlers exposed to higher prenatal cortisol and/or maternal anxiety showed greater negative reactivity (Davis et al., 2007; de Weerth, van Hees, & Buitelaar, 2003), more behavioral agitation such as crying and deficits in attention (Gutteling, de Weerth, Willemsen-Swinkels, et al. 2005; Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2002; O'Connor, Heron, Goldin, & Glover, 2003), and slower rates of behavioral recovery in response to stressful stimuli (Davis et al., 2011). Taken as a whole, the programming of prenatal glucocorticoid exposure can enduringly influence both later-life normative HPA functioning and individual HPA and behavioral responses to stressful stimuli.

The exact nature of the relationship between prenatal programming effects linked to gestational glucocorticoid exposure and later-life outcomes is unclear given the moderating influences of varying postnatal environments on these same outcome measures. Like prenatal stress and glucocorticoid exposure, poor or insecure mother-infant attachments, parental maltreatment, or early social deprivation influence the HPA response to stress (Capitanio, Mendoza, Mason, & Maninger, 2005; Dettling, Parker, Lane, Sebanc, & Gunnar, 2000; Gunnar & Donzella, 2002; Koch, McCormack, Sanchez, & Maestripieri, 2012; Tarullo & Gunnar, 2006), alter behavioral trajectories (Corcoran et al., 2012; Dettling, Feldon, & Pryce, 2002a; Dettling, Feldon, & Pryce 2002b; Suomi, 1997), and lead to vulnerabilities to mood and anxiety disorders in both human and non-human primates (reviewed in Heim & Nemeroff, 2001; Pryce et al., 2005). Importantly, in contrast to early social deprivation, some evidence shows that socially and physically enriching environments can offset or reverse the effects of prenatal glucocorticoid exposure or stress on later-life

reactivity and behavior. In particular, peripubertal social and physical environmental enrichment reversed the effect of maternal separation on HPA and behavioral responses to stress in rats (Francis, Diorio, Plotsky, & Meaney, 2002; Morley-Fletcher, Rea, Maccari, & Laviola, 2003). Thus, an infant's early social and physical environment has the potential to moderate the effects of fetal programming through gestational glucocorticoids. The extent to which early socially enriching environments may offset gestational programming of later-life neuroendocrine and behavioral reactivity to stressful stimuli is not as thoroughly explored, especially in non-human primates.

Marmosets are a highly social primate species that show sensitivity to early social environments and reach maturation quickly (Birnie et al., 2013; Dettling et al., 2002a,b; Pryce, Dettling, Spengler, Spaete, & Feldon, 2004). The development of HPA responses and stress reactivity to a social separation paradigm in marmosets are characterized by high inter-individual variability, but individual patterns of stress reactivity are highly stable within individuals across broad developmental stages, suggesting a "trait" like response to stressful stimuli (French et al., 2012). Consequently, marmosets serve as an exemplary nonhuman primate model for examining the effects of prenatal glucocorticoid exposure and varying social conditions during development on early adult outcomes in stress reactivity and behavior, and linking these early-life experiences with trait-like responses to stressful stimuli. One of the primary gaps in the literature is the investigation of natural variation in glucocorticoid exposure across gestation and sequelae of glucocorticoid exposure longitudinally in order to determine the extent and longevity of the fetal programming on both neuroendocrine and behavioral outcomes. Furthermore, it is unclear if enriching postnatal experiences moderate the influence of gestational cortisol exposure on later-life HPA and behavioral responses to stressful stimuli. Therefore, the thrust of this paper is to explore the relationship between variation in cortisol exposure across gestation and subsequent HPA response characteristics, and to assess whether variation in early social experiences (in this case, social play) can moderate the effects of glucocorticoid exposure. If gestational exposure to glucocorticoids alters early programming of the HPA axis, then we would expect elevated cortisol and greater behavioral agitation in response to a standardized stressor in marmoset offspring exposed to higher levels of gestational glucocorticoids. Moreover, if enhanced socially rich environments, by increased juvenile social play behavior, offset or attenuate the outcomes associated with increased prenatal cortisol exposure, then we expect individual marmosets who initiate and receive more social play will have an attenuated HPA and behavioral response to social stress beyond the potentially resultant early-life programming from the gestational glucocorticoid exposure. Finally, we seek to explore if the nature of any of these effects depends on the gestational timing of cortisol exposure, and the age and sex of the offspring.

METHODS

Subjects

The study included longitudinal data collected from 28 white-faced marmosets (*Callithrix geoffroyi*) born into the colony at the Callitrichid Research Center at the University of Nebraska at Omaha. Mothers (maternal age range: 2.22–9.13 years) were housed in large

breeding enclosures with an unrelated adult male, and offspring were housed with family units including parents and siblings. Colony rooms were maintained at 19.7–22.1°C and on a 12:12 light-dark cycle. Wire-mesh enclosures varied in size depending on the number of offspring in the breeding group, with a minimum of 0.8 m^3 per animal. Enclosures included branches, rope vines, nest boxes, and various enrichment items. All animals were fed twice each day, first at 0800–0900 hr and then later in the afternoon at 1300–1500 hr. The Institutional Animal Care and Use Committee at the University of Nebraska at Omaha approved all animal use procedures (IACUC 07-033-05-FC) and these procedures followed all ethical guidelines and principles endorsed by the International Society for Developmental Psychobiology. Further details of animal housing and husbandry have been previously reported (Schaffner, Shepard, Santos, & French, 1995).

Homecage Urine Collection From Pregnant Mothers and Offspring First-Void Samples

First-void urine samples from breeding females and offspring were collected using noninvasive collection techniques (French et al., 1996). One to three times a week, we collected samples between 0700 and 0830 hr by holding aluminum pans under a stream of urine in exchange for a valued food reward. We ensured only one individual's urine was present in each pan, and the timing of urine collection coincided with the waking of marmosets. Urine samples were transferred into individual microcentrifuge tubes, and the samples were centrifuged at 5,000 rpm for approximately 2 min to separate the urine from any sediment contamination. The urine was transferred into a clean test tube and stored at −20°C until assayed. Urine sampling reflects total circulating hormones levels since the last time the bladder was emptied, and first-void urine samples are an accurate and readily available representation for the composite daily average of circulating hormones (French et al., 1996).

Homecage Play Behavior Observations

We collected play behavior data on offspring between the ages of 5–10 months. This time period coincides with the juvenile period in marmosets in which a well-developed and highly variable repertoire of play behaviors is exhibited (Yamamoto, 1993). We collected two to five 20-min observations per week for each offspring. A trained observer sat approximately one meter from the home cage with a laptop computer and recorded behaviors with Observer software (Noldus Technologies, Houston, TX). All-occurrences of behaviors for play-bout frequencies were scored among all family members. For each play bout, we scored three components of rough-and-tumble play: initiate play, receive play, and overall play (composite of initiate and receive). Successful play initiations were combined to give a composite "initiate play" score, and the animal that engaged in rough- and-tumble play with the play initiator was given a "receive play" score. Overall, social play was the combined initiated and received play scores. A new play bout was scored only if 5 s elapsed without a play bout between interactants. See Birnie, Hendricks, Smith, Milam, and French (2012) for a full ethogram of play behaviors.

Social Separation Stressors

Marmosets underwent social separation stress challenges at 6, 12, and 18 months of age. Data for the current study were obtained from the same population of animals described in

previous reports on stress reactivity from our lab (Birnie et al., 2013; French et al., 2012). First-void urine samples were collected on the day prior to, and immediately before separation on the day of the stressor manipulation. At 0900 hr, the marmoset was removed from its home cage and placed in a small wire mesh separation cage measuring 0.5 m^3. Separation cages were placed in a separate, quiet room some distance away from the home cage. These cages were furnished with a food dish and a water bottle filled with diluted apple juice to facilitate urination. Animals remained in the separation cage until 1700 hr (total of 8 hr of separation). This type of social separation has been shown to induce a measurable and significant increase in urinary glucocorticoid excretion compared to normal circadian variation (Johnson et al., 1996; Rukstalis & French, 2005; Smith & French, 1997). A final first-void urine sample was collected between 0700 and 0830 hr on the day after the social separation paradigm.

During the social separation, we attempted to collect urine from the isolated marmoset every hour from 1000 to 1700 hr. Clean polyethylene sheets were placed underneath the separation cage, and sheets were replaced after each hourly collection. Not all marmosets provided samples at each hourly interval, but samples were collected on at least every 2-hr interval for all marmosets.

During the first 60 min of the social separation challenge, animals were observed by trained technicians to evaluate behavioral responses to the initial separation challenge. Behavioral measures considered in this study included locomotion (the frequency the individual moved across the six sides of the isolate cage), and cage manipulations (the frequency the individual grabbed, bit, or manipulated in any way parts of the cage, or items connected or near to the cage, such as the plastic lining, food trays, or water bottles). Increases in these behaviors are associated with increases in stress or anxiety associated with the social separation challenge (French et al., 2007).

Pregnanediol-3-Glucuronide (PdG) Enzyme Immunoassay (EIA)

Determination of conception and gestational timing was evaluated by monitoring excreted levels of the progesterone metabolite, PdG, in breeding females. Urinary PdG levels were monitored by EIA following the protocol established for marmosets (for details, see French et al., 1996; Mustoe, Jensen, & French, 2012). Inter-assay coefficients of variation (CV), determined from high and low concentration pool samples ran on each plate, were 22.0% and 6.1%, respectively. Intra-assay CVs for the high and low pools 28.0% and 6.8%, respectively. Variation in fluid intake and output was indexed by measuring concentrations of urinary creatinine (Cr). The creatinine assay utilized a modified Jaffe end-point assay, previously described and validated for use in marmosets (French et al., 1996). Pregnancy was defined as PdG concentrations >10 μg/mg Cr for a minimum of 30 days, and the estimated day of conception was defined as the first sample proceeding the beginning of the 30-day consecutive samples of PdG concentrations >10 μg/mg Cr (Mustoe et al., 2012).

Cortisol EIA

We measured urinary cortisol concentrations in samples collected from pregnant mothers in their homecage and from samples collected from offspring during social separation

challenges via an EIA previously developed and validated for marmoset urine (Smith & French, 1997). Microtiter plates were coated with rabbit anticortisol antibody and 50 μl of standard and urine sample (diluted 1:6,400 in distilled, deionized water) was added. Standards were serially diluted in distilled and deionized water, with a lower-bound sensitivity of 7.8 pg/well. Labeled cortisol (horseradish peroxidase, HRP) was added to each well, and the plates were incubated for 2 hr. We separated free from bound hormone in each well by washing the plate four times with saline-buffered detergent solution (Tween 20; Sigma Chemicals, St. Louis, MO) and then added 100 μl of substrate (ABTS and H_2O_2) and allowed the plate to develop until the B_0 wells reached an optical density of approximately 1.0. The sample concentration was determined by interpolating optical densities with a four-parameter sigmoidal fit function. Inter-assay coefficients of variation (CV), determined from high and low concentration pool samples ran on each plate, were 12.28% and 14.90%, respectively. Intra-assay CVs for the high and low pools 8.94% and 9.78%, respectively. To minimize procedural variability, all samples for a single individual were assayed at the same time whenever possible. As with PdG, urinary cortisol concentrations in each sample were adjusted for variable fluid intake and output by correcting for urinary creatinine concentration.

Gestational Cortisol Exposure (GCE)

First-void urine samples were collected two to five times per week and first and third trimester durations in pregnant females were determined by individual pregnancy gestation length divided into thirds where pregnancy and conception was determined using the PdG EIA procedure described above. Cortisol concentrations for first-void urine samples were determined using the Cortisol EIA procedure discussed above. Thus, GCE for the first and third trimesters were estimated by the average cortisol concentrations across the complete duration of the first and third trimester for each pregnancy.

Data Analysis

Overview of Model—Multi-level modeling (MLM) was used to assess the relationship of GCE, juvenile social play, and offspring sex on HPA reactivity across developing marmoset offspring. MLM functions similarly to traditional hierarchical regression, but one of the primary advantages of MLM over traditional hierarchical regression is the ability to account for non-independence of longitudinal data (Raudenbush and Bryk, 2002). Non-independence of data in this study results from the same individuals measured repeatedly across time and age. Specifically, cortisol data (the outcome or dependent variable) is measured across time during the social separation challenge (three first void urine samples across three consecutive days, and 8 hr of urine samples across the separation challenge on Day 2), and across age (social separation challenge occurred at 6, 12, and 18 months of age). We were interested in whether basal cortisol levels and levels of cortisol reactivity ($n_{\text{samples}} = 865$) were influenced by time ($n_{\text{timepoints}} = 924$), age ($n_{\text{marmosets} \times \text{age points}} = 84$), and, most importantly, of individual offspring ($n_{\text{marmosets}} = 28$). Factors for individual marmosets included offspring sex, offspring GCE, and the amount of juvenile social play between 5 and 10 months of age. Consequently, these data are represented in three levels: Cortisol (the dependent variable) is accounted for by both the linear and curvilinear (time^2) change in time during the social separation challenge (level 1); maturation or change in age from 6, 12,

and 18 months postnatal (level 2); and offspring sex, GCE, and levels of juvenile social play (level 3). Information about higher levels, such as individual differences in levels of exposure to cortisol during gestation and levels of early juvenile social play were used to predict the intercept (individual levels of basal cortisol) and slope (individual levels of cortisol reactivity), that is, change in cortisol reactivity during the stressor and change in cortisol levels across age. More specifically, we evaluated whether GCE or juvenile social play predicted HPA activity, that is, basal cortisol intercept and cortisol reactivity slope, and the maturation (change across age) of these factors across the developing marmoset. It is worth noting that multiple offspring from the same litters were analyzed at the individual level rather than the litter level to account for greater individual postnatal variation in juvenile social play and HPA characteristics. Twin offspring from the same litter were treated as having identical GCE. All variables in the MLM models other than time are grand centered. Finally, correlations between GCE and individual behaviors during the social separation challenge such as locomotion and cage manipulations and juvenile social play were tested. Only first and third trimester analyses were included to emphasize the notion of early or later gestational effects on postnatal outcomes. All multi-level analyses were conducted using HLM version 6.08, and bivariate correlations were calculated using SPSS 19.0.

Justification of Model—The unconditional model, where no variables were regressed on urinary cortisol concentrations following the social separation challenge, resulted in an intra-class correlation wherein 67.2%, 15.2%, and 17.6% of the variance in cortisol concentrations was attributable to variability over time (level 1), variability across age (level 2), and variability between individual factors including prenatal cortisol exposure, juvenile social play, and offspring sex (level 3), respectively. This highlights the sources of variation in cortisol, and the final estimation of variation in cortisol concentrations following the social separation challenge resulted in a significant amount of variability left to account for in differences in maturation of the marmoset across age ($\chi^2_{(56)} = 184.49$, $p < .001$) and differences in basal cortisol concentrations and cortisol reactivity explained by GCE, juvenile social play, and offspring sex ($\chi^2_{(27)} = 96.34$, $p < .001$). This indicates that variations in GCE, juvenile social play, and offspring sex are related to basal cortisol and cortisol reactivity above and beyond variance accounted for by the change in cortisol over the duration of the stressor (time) and marmoset age alone (time of the stressor: 6, 12, or 18 months). Cortisol concentrations significantly increased in response to the social separation stressor (β's > .95, all p's < .001) indicating the social separation paradigm elicits a robust glucocorticoid stress response. Basal concentrations of cortisol and cortisol reactivity decreased across age (β's > .29, all p's < .003). The change from pre- to post-stressor basal concentrations of cortisol were positively associated with cortisol reactivity ($\beta = 0.08$, $p < .001$). However this change was not significantly accounted for by variance in GCE, juvenile social play, and offspring sex ($\chi^2_{(27)} = 11.56$, $p > .50$), so pre- and post-basal concentrations were treated as an overall basal cortisol concentration. A summary of the normative cortisol response to the social separation challenge and the development of stress reactivity across age are described in French et al. (2012).

RESULTS

Association Between GCE and HPA Activity

First Trimester GCE—Variation in marmoset GCE affected multiple components of HPA function in prepubertal, peripubertal, and postpubertal marmosets. Overall, first trimester GCE predicted differences in cortisol concentrations above and beyond the effects of time, age, and sex ($\Delta\chi^2_{(6)} = 24.59$, $p = .001$). Higher GCE during the first trimester was associated with elevated offspring basal cortisol concentrations ($\beta = .23$, $p < .001$), and increased offspring cortisol reactivity, that is, change in cortisol over the duration of the stressor (linear: $\beta = .24$, $p = .02$; curvilinear: $\beta = -.18$, $p = .02$; see Fig. 1). The strength of the association between GCE and cortisol reactivity during the stressor decreased significantly across age (linear: $\beta = -.20$, $p = .01$; curvilinear: $\beta = .14$, $p = .05$; see Fig. 1), and these data suggest that the relationship between gestational cortisol and later-life HPA activity weakens throughout development. Overall, increased first trimester GCE is associated with increased basal cortisol concentrations, increased cortisol reactivity to stressful stimuli, and greater reduction in cortisol reactivity across developing offspring.

Third Trimester GCE—Third trimester GCE did not predict differences in cortisol concentrations above and beyond the effects of time, age, and sex ($\Delta\chi^2_{(6)} = 3.59$, $p > .50$), suggesting that third trimester GCE was not a good predictor of HPA functioning. Third trimester GCE was not associated with offspring basal concentrations of cortisol in marmosets or with changes in cortisol reactivity during the social separation (β's < .11, all $p > .32$). As with first trimester GCE, there was a decrease in the association between third trimester GCE and offspring cortisol reactivity across age ($\beta = -.10$, $p = .05$), but this effect size was modest, and the overall association between late gestational cortisol and cortisol reactivity was not significant. It appears that the association between GCE and later-life basal cortisol concentrations and cortisol reactivity is stronger during first trimester GCE than third trimester GCE (Fig. 1 and Tab. 1).

Offspring Sex Differences—There were no major sex differences in the relationship between GCE and HPA activity. Offspring sex does not predict differences in cortisol concentrations above and beyond the effects of time and age ($\Delta\chi^2_{(6)} = 3.05$, $p > .50$). There was a trend for females to show a stronger reduction in basal cortisol across development than males ($\beta = -.03$, $p = .06$). However, this relationship is not significantly strengthened when controlling for both first and third trimester GCE (first: $\beta = -.06$, $p = .08$; third: $\beta = -.04$, $p = .07$). Overall, when controlling for the effect of GCE, there was no association between sex and marmosets' cortisol reactivity in response to the stressor across development.

Association Between GCE and Behavioral Phenotypes—First trimester GCE was negatively correlated with offspring levels of juvenile social play, while third trimester GCE was not significantly correlated with offspring levels juvenile social play (Fig. 2). This relationship, like the relationship between gestational cortisol and HPA activity, shows that first trimester GCE is more strongly associated with juvenile social play in marmosets than

third trimester GCE. First trimester GCE is positively correlated with locomotion behaviors at 6 months of age but not at 12 or 18 months of age (Fig. 3a–c). Likewise, third trimester GCE was positively associated with locomotion at 6 months of age but not 12, or 18 months of age (Fig. 3d–f). Conversely, cage manipulations during the social separation stressor at any age were not significantly associated with both first and third trimester GCE (all r's < .21, p's > .28). The relationship between GCE and behavioral responses to the social separation challenge appear early in marmoset maturation (6 months) and only for locomotive behaviors. These data are aligned with the endocrine data showing that first trimester GCE is associated with cortisol activity, especially for prepubertal marmosets. However, unlike for patterns of cortisol activity, the patterns of behavioral responses during the social separation stressor are not specifically associated with the timing of GCE.

Association Between Juvenile Social Play and HPA Activity

Juvenile social play significantly affected normative HPA activity and HPA responses to stress throughout development. Variation in juvenile social play accounted for differences in HPA functioning above and beyond the effects associated with GCE ($\Delta\chi^2_{(6)} = 12.91$, $p = .04$), indicating that social play is an important predictor of HPA parameters. Specifically, marmosets who engaged in higher social play showed decreased basal concentrations of cortisol ($\beta = -.18$, $p = .02$), and showed a marginally greater reduction in cortisol reactivity as they aged ($\beta = -.19$, $p = .08$; Fig. 4 and Tab. 2). In light of the finding that the relationship between GCE and cortisol reactivity decreases significantly across development, these data suggest a potential marginal attenuation in stress-induced HPA activity later in development at 12 and 18 months of age for marmosets who show high juvenile social play. For individual marmosets in the highest and lowest quartiles, the relationship between GCE and cortisol reactivity is marginally reduced across age, where the difference in cortisol reactivity is greatest in 6-month-old marmosets. Conversely, the relationship between juvenile social play and cortisol reactivity is augmented across age after controlling for GCE, where the difference in cortisol reactivity is greatest in 18-month-old marmosets. These data suggest that early social play is an important variable in the shaping of adolescent HPA development, especially when accounting for GCE as social play significantly improves the overall model prediction above and beyond the effects of GCE.

DISCUSSION

The findings presented here document how naturally occurring variation in exposure of marmoset fetuses to cortisol of maternal origin across gestation is associated with multiple features of endocrine and behavioral development. Specifically, we found that marmosets with increased first trimester GCE exhibited increased basal cortisol concentrations and higher cortisol reactivity in response to a social separation stressor, and this relationship between first trimester GCE and cortisol reactivity weakened across development. Moreover, this study is the first to indicate that this gestational cortisol programming of HPA activity in marmosets is potentially attenuated by increased participation in juvenile social play after controlling for the effects of time, age, sex, and GCE. Marmosets that engaged in increased levels of social play between 5 and 10 months of age show decreased basal concentrations in cortisol and a marginally greater reduction of the cortisol response to the social separation

stressor as the marmosets develop. Lastly, our findings highlight that the relationship between GCE and later-life HPA outcomes appear more strongly linked to variation in maternal cortisol in early gestation rather than late gestation.

Gestational Cortisol Exposure and Later-Life HPA Activity

It is expected that basal and reactive functioning of the HPA axis would be sensitive to variation in cortisol during pregnancy. The “fetal programming hypothesis” posits that differences in behavioral and health outcomes can be attributed to differences in fetal environments (Barker, 1990, 1998). It is likely that intrauterine influences on the developing fetus would serve as an adaptive preparation for the fetus’ subsequent environment and development. Since cortisol is an end-product of the HPA response to stress and arousal, mothers who exhibit increased cortisol during pregnancy are more likely to be mothers in environments where increased arousal, anxiety, and/or stress are more frequently experienced. Consequently, the more arousing or stressful the environment is during the pregnancy, the more likely a reactive HPA system may be beneficial for offspring. The effects associated with increased cortisol exposure or stress during pregnancy, such as increased anxiety, attentional distractions, impulsivity, and altered HPA functioning could serve adaptive functions in highly stressful or arousing environments, such as increased vigilance, alertness, or sensitivity to dangerous or stressful signals (reviewed in Glover, 2011; Matthews, 2002). Here, we reveal that marmoset mothers that showed greater increases in cortisol during early gestation were more likely to have offspring that showed greater basal cortisol levels and greater reactive cortisol responses to stressful stimuli. Furthermore, we would additionally expect alterations in behavioral phenotypes to match the reactive endocrine responses. First, we found a relationship between increased first trimester GCE and decreased social play. Second, we found a relationship between increased overall GCE and increased stressor-induced locomotion. Together, these data suggest that marmosets’ GCE are associated with behavioral phenotypes typical of a more anxious and reactive manner, at least early in life.

We found that endocrine responses to stress were only associated with variation during first trimester, while the behavioral response to stressors (locomotion) was associated across the entire gestation but only in young marmosets and not adolescent marmosets. Previous research has linked early GCE to human infant behavioral responses and later GCE to infant cortisol responses (Davis et al., 2011; Davis and Sandman, 2010). Our findings suggest that later-life marmoset basal cortisol concentrations and cortisol reactivity are more sensitive to variation in first trimester GCE where sensitivity to alterations in brain development and organization is higher. Conversely, behavioral phenotypes like social play and locomotion responses to the stressor were also correlated with early GCE, but, also, later GCE (for locomotive behaviors in young marmosets). However, this effect is less permanent in that this relationship does not persist into adolescence. Given the varying levels of cortisol exposure, the nature of the gestational stressors, species differences in gestational physiology, length of gestation, and timing of key developmental milestones, the effect of specific timing on GCE on postnatal outcomes can range considerably, but in many cases, these effects are malleable based on the quality of postnatal social interactions.

Postnatal Reprogramming of HPA Activity

Though marmoset's normative and stress-induced HPA function was associated with increased GCE, it remains foremost to consider that postnatal developmental plasticity may play a pivotal role in the overall development trajectory of offspring. First, fetal programming may not be a completely satisfactory conclusion without evidence that the effects on offspring HPA functioning persist into adolescence and adulthood. Given that the normative HPA functioning undergoes considerable maturation postnatally (French et al., 2012; Gunnar, Brodersen, Krueger, & Rigatuso, 1996; Pryce, Palme, & Feldon, 2002), firm conclusions of postnatal effects attributed to GCE potentially underestimate the influences associated with early social and physical environments, especially for adolescent and early adulthood behavioral and endocrine phenotypes. Our data support this conclusion, as the association between GCE and HPA reactivity significantly declines from 6-month-old marmosets (juvenile) to 18-month-old marmosets (young adults). This suggests that either the first trimester GCE programming of HPA activity does not persist into adolescence, and is therefore not permanent, or environmental factors, such as social interactions, may reprogram the HPA activity independently of GCE.

Juvenile social play in marmosets appears to play an important role in adolescent HPA activity regardless of individual GCE. Marmosets' patterns of basal HPA activity are significantly associated with their early social environment (social play) and their change in HPA responses to stressors across development are marginally associated with their early social environment (social play). This is congruent with other findings that early social environments may also program offspring HPA and behavioral responses to stressful stimuli (Green, Barnes, & McCormick, 2012; Liu et al., 1997). Specifically, adolescent rats that were prenatally stressed and received physical environmental enrichment showed increased play and a reduced glucocorticoid response to stressful stimuli, while rats that were not prenatally stressed did not show a reduction in glucocorticoid reactivity, though, interestingly, they still showed an increase in social play (Morley-Fletcher et al., 2003). Moreover, Francis et al. (2002) showed that only rat infants who were separated from their mothers for periods postnatally were positively impacted by peripubertal environmental enrichment, while rats that did not receive maternal deprivation were relatively unaffected. This suggests that enriching environments are effective at alleviating heightened HPA reactivity, such as instances commonly observed following high GCE or deprived early social environments. Thus, in both rats and non-human primates, environmental enrichment such as toys and social play may operate as important moderating variables in either attenuating or even reversing the fetal programming associated with increased gestational glucocorticoids, and these, themselves, can have potentially long-lasting changes on HPA and behavioral outcomes.

The response to stressors in rats and primates is intimately tied to social play. This relationship appears to be somewhat paradoxical though. In some cases, immediately following an acute stressor play levels in adolescent rats are completely suppressed, but in cases following repeated restraint stressors, the negative impact of stress on play is only short-term following termination of the stressor (Klein, Padow, & Romeo, 2010). Conversely, play can aid in the response to stressors. Specifically, marmoset scratching, a

behavioral indicator of stress or agitation, was reduced following increased play, while levels of play remained unchanged with or without scratching (Norscia & Palagi, 2011). This finding suggests that play reduces behavioral indicators of stress more than do the onset of stressors influence long-term play. Moreover, when play is deprived during adolescence, rats show decreased social activity as adults and their corticosterone levels remained elevated longer following social defeat (van den Berg et al., 1999). The juvenile to adult transition appears to be an especially sensitive period for the development of stress reactivity (Romeo, 2010). Our findings support this notion, since, in relation to social play, it is the marmosets during and following puberty that show the greatest differences in HPA activity. Because play seems to play an important role in countering the stress response, increased social play during this sensitive period may condition the HPA to be less reactive to typical or mild stressors.

It is important to consider whether gestational and early postnatal programming affect offspring HPA activity and behavior similarly or differentially. In this study, we found that increased GCE was associated with increases in both stress-induced HPA activity and locomotion in young marmosets, and at this early stage, there appears to be a co-activation of GCE on HPA and behavioral activity. Later in development, however, increased GCE was still associated with heightened HPA activity, but no longer had an effect on stressor-induced behaviors. While overall activation of behavioral and endocrine responses to the social separation challenge are to some extent independent, (Taylor, Mustoe, & French, 2013), we documented fetal programming on both stressor-induced locomotive behavior and endocrine responses for 6-month-old marmosets. However, the effect of GCE on behavioral responses to stressors is much weaker than its effect on endocrine response to stressors, and weakens further across development. This difference educes the nature of the relationship between environmental influences such as the social environment or endocrine influences such as puberty and their alteration on the trajectory of HPA development. While GCE and the development of the HPA stressor response system most likely use similar mechanisms, behavioral adaptations are more sensitive to environmental and experiential influences (Coe, Glass, Wiener, & Levine, 1983; Levine, Wiener, & Coe, 1993). Furthermore, locomotive behaviors in response to a stressor do not stabilize until puberty in marmosets (Taylor et al., 2013), and this suggests that other behavioral adaptations, like engaging in play with others, could play an important role in modifying responses to stressors. While more work is essential to uncover the specific relationship between postnatal social environments and behavioral responses to stressors, factors like gestational programming, postnatal influences, and changing behavioral repertoires may potentially reflect three different adaptation strategies. First, gestational programming provides a biological/organizational adaptation to the environment; second, positive social interactions modify stress reactivity to regulate social and physical interactions with the environment; and third, behavioral adaptation provides a response strategy via learning which may be tailored to specific situations and experiences.

CONCLUSIONS

These findings are among the first to document the relationship between natural variation of cortisol exposure across the entire pregnancy with behavioral and endocrine outcomes both

before and during adolescence in non-human primates. Having multiple time points of both GCE and longitudinal developmental outcomes is a primary strength of this study. In addition, the use of a non-human primate model of a highly social species such as the marmoset can provide valuable insight as to the degree and nature of the moderation of early-life social experiences on both endocrine and behavioral later-life outcomes. However, some limitations are worth mentioning. The measures of GCE are indirect, and the degree to which the cortisol influenced fetal and placental physiology was not tested. Additionally, other postnatal sources of cortisol delivery to the offspring, such as lactation have been shown to influence behavioral outcomes (Hinde, 2013). Finally, the interdependence of the offspring's relationship with their mothers, fathers, siblings, and other social partners, which may influence the response to arousing stimuli (Birnie et al., 2013; Hennessy, Kaiser, & Sachser, 2009; Parker & Maestripieri, 2011), or many other sources of positive social interactions, were not accounted for directly in this analysis. The nature of these limitations warrants further investigation. Here, we suggest that the adaptability of HPA functioning can be viewed as resulting in both positive and negative outcomes, but, importantly, the adaptability should be viewed in the context of the offspring's current environmental demands, and HPA functioning, to some extent, exhibits a degree of malleability across development. Finally, these findings suggest two important conclusions. First, increased early GCE exposure is related to more reactive behavioral and endocrine phenotypes. Second, increased levels of juvenile social play are associated with decreased basal levels of cortisol in adolescence and a marginal decrease in cortisol reactivity across development regardless of the offspring's GCE exposure. Thus, socially enriching postnatal environments can shape the development of later-life HPA functioning either in spite of or apart from the prenatal effects associated with increased fetal exposure to glucocorticoids.

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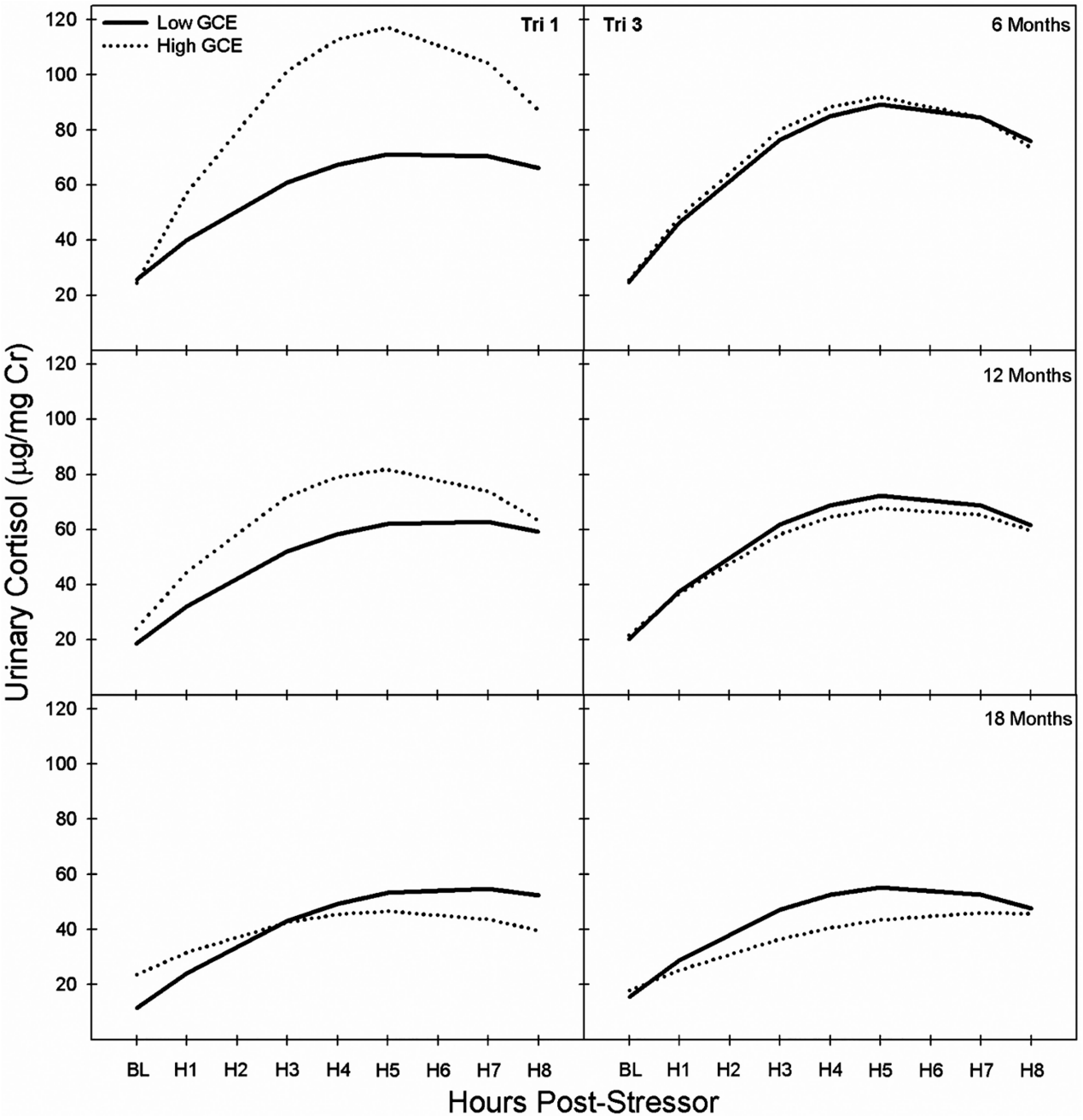

FIGURE 1.

Modeled graph of the association between 1st and 3rd trimester (Tri) gestational cortisol exposure (GCE) on marmoset offspring's pre- and post-stressor basal (BL) and cortisol (µg/mg Cr) reactivity, that is, change in urinary cortisol over time in H (hours), at 6, 12, and 18 M (months) of age after controlling for offspring sex and individual differences in HPA activity across age and time. High and low GCE reflect modeled cortisol reactivity change over time for upper and lower quartiles of GCE.

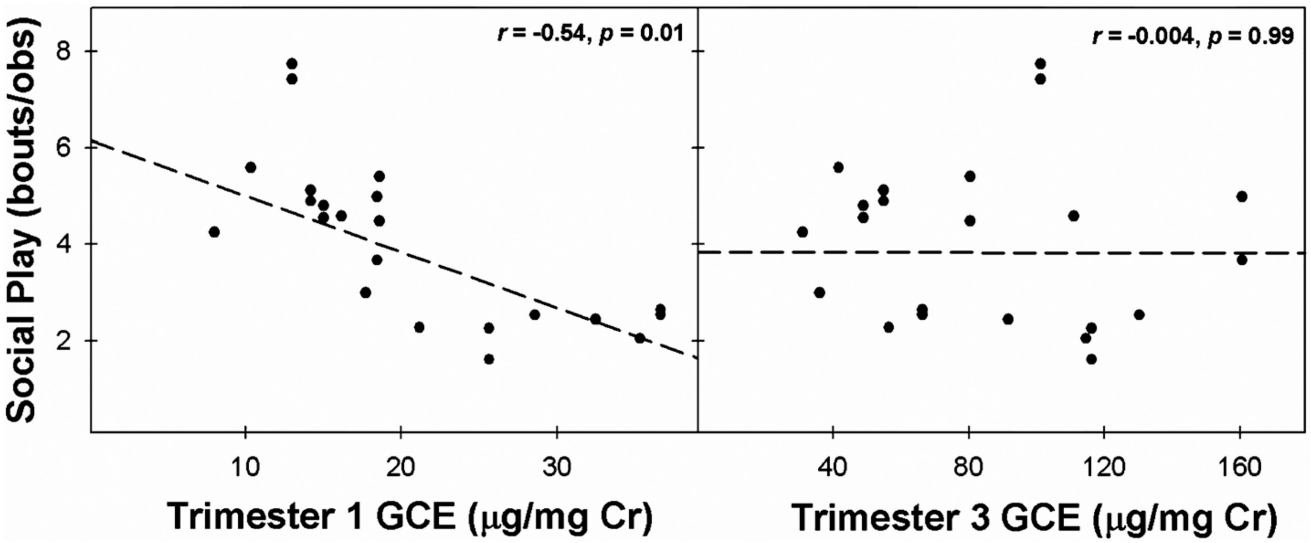

FIGURE 2.

Association between first and third trimester gestational cortisol exposure (μg/mg Cr) (GCE) and levels of social play (number of play bouts/observation) in individual juvenile marmosets.

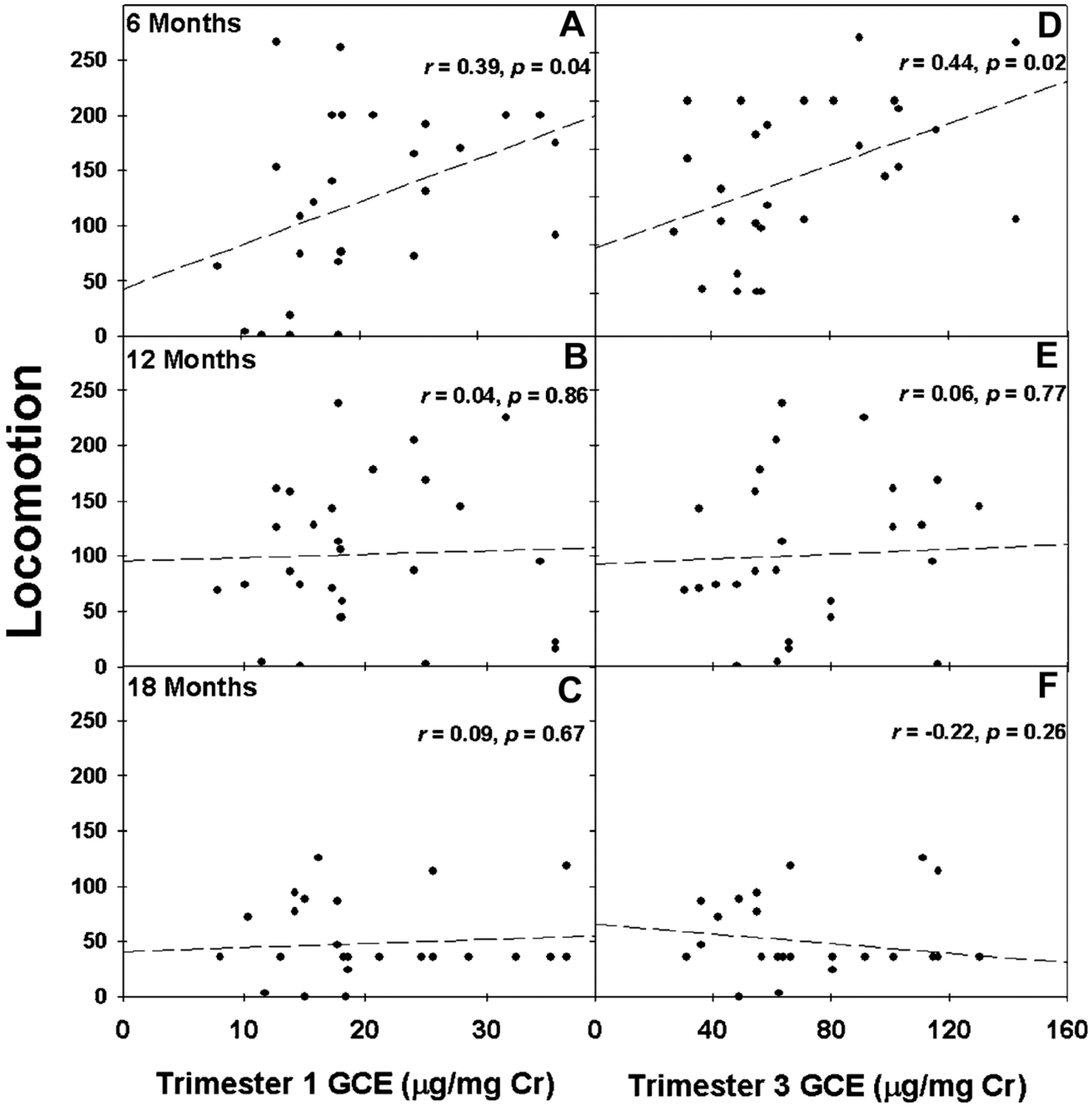

FIGURE 3.

Association between first and third trimester gestational cortisol exposure (μg/mg Cr) (GCE) with individual locomotion behavioral responses (frequency of locomotion) during the social separation stressor at 6, 12, and 18 months of age. A–C reflect first trimester GCE and D–F reflect third trimester GCE across 6, 12, and 18 months of age.

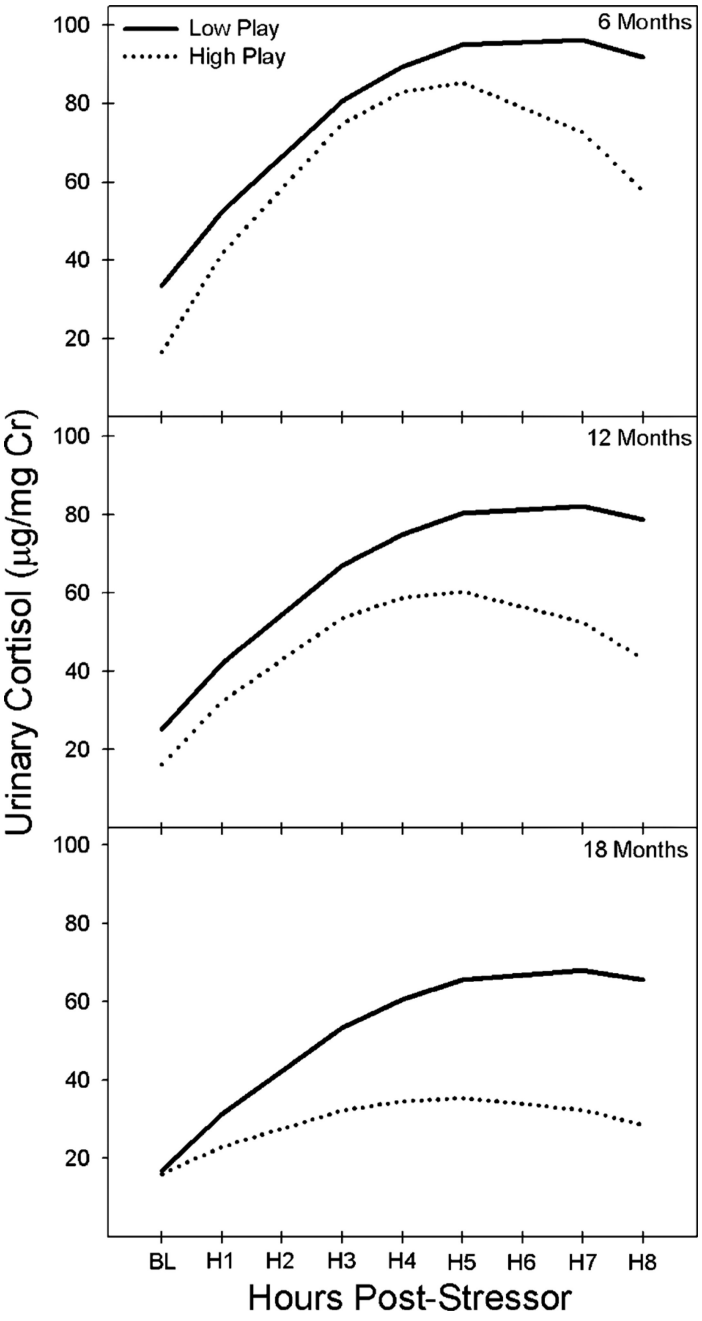

FIGURE 4.

Modeled graph of the association between levels of juvenile social play (number of play bouts/observation) on marmoset offspring's pre- and post-stressor basal (BL) and cortisol (µg/mg Cr) reactivity, that is, change in cortisol over time in H (hours), at 6, 12, and 18 M (months) of age after controlling for, GCE, offspring sex, and individual differences in HPA activity across age and time. High and low play reflect modeled cortisol reactivity change over time for upper and lower quartiles of levels of juvenile social play.

Table 1

Association Between Offspring GCE and Normative and Stress-Induced HPA Activity Across Development

	1st Trimester				3rd Trimester			
Predictor	***β***	***b***	***SE***	***t*-Ratio**	***β***	***b***	***SE***	***t*-Ratio**
Basal CORT								
Sex	0.07	3.32	2.34	1.42	−0.01	0.48	2.65	0.18
GCE	0.24	0.54	0.11	4.70**	−0.06	−0.002	0.04	−0.04
Basal CORT across age								
Sex	−0.06	−0.73	0.40	−1.84ψ	−0.04	−0.77	0.41	−1.89ψ
GCE	−0.10	0.02	0.02	0.76	−0.03	0.002	0.005	0.44
CORT reactivity								
Linear								
Sex	0.02	0.48	3.31	0.15	−0.06	−1.69	3.45	−0.48
GCE	0.24	0.40	0.15	2.61*	−0.11	−0.04	0.04	−1.01
Curvilinear								
Sex	0.01	0.02	0.37	0.04	0.06	0.22	0.38	0.59
GCE	−0.18	−0.04	0.02	−2.45**	0.08	0.004	0.004	0.90
CORT reactivity across age								
Linear								
Sex	−0.01	−0.05	0.39	−0.17	0.04	0.22	0.43	0.52
GCE	−0.20	−0.07	0.02	−2.72**	−0.10	−0.01	0.003	−2.08*
Curvilinear								
Sex	−0.01	−0.001	0.04	−0.06	−0.04	−0.03	0.05	−0.53
GCE	0.14	0.006	0.003	2.09*	0.10	0.001	0.001	1.69

Note: t-Ratios are of unstandardized coefficients. *SE*, standard error.

ψ $p < .10$,

* $p < .05$,

** $p < .01$ and are two-tailed.

GCE refers to gestational cortisol exposure, and CORT refers to cortisol. Model examined the relationship between GCE and offspring sex, on different HPA parameters.

Table 2

Association Between Juvenile Social Play and GCE on Normative and Stress-Induced HPA Activity Across Development

	Final Model			
Predictor	***β***	***b***	***SE***	***t*-Ratio**
Basal CORT				
Sex	−0.02	−0.09	2.83	−0.03
GCE	0.13	0.27	0.16	1.72 $^{\Psi}$
Play	−0.18	−1.61	0.63	−2.57 *
Basal CORT across age				
Sex	−0.07	−0.23	0.41	−0.55
GCE	−0.10	0.06	0.03	2.00 $^{\Psi}$
Play	−0.02	0.24	0.12	2.05 $^{\Psi}$
CORT reactivity				
Linear				
Sex	0.01	0.32	4.95	0.06
GCE	0.23	0.39	0.28	1.41
Play	−0.01	−0.07	1.19	−0.06
Curvilinear				
Sex	−0.04	−0.12	0.53	−0.24
GCE	−0.23	−0.05	0.03	−1.61
Play	−0.08	−0.07	0.14	−0.48
CORT reactivity across age				
Linear				
Sex	−0.10	−0.55	0.37	−1.50
GCE	−0.31	−0.10	0.04	−2.95 **
Play	−0.19	−0.23	0.13	−1.79 $^{\Psi}$
Curvilinear				
Sex	0.07	0.05	0.05	0.97
GCE	0.22	0.01	0.004	2.46 *
Play	0.15	0.03	0.02	1.51

Note: t-Ratios are of unstandardized coefficients. *SE*, standard error.

$^{\Psi}p < .10$,

$^{*}p < .05$,

$^{**}p < .01$ and are two-tailed.

GCE refers to gestational cortisol exposure, and CORT refers to cortisol. Model examined juvenile social play, GCE, and offspring sex on different HPA parameters.