Adrenocortical Interdependence in Father-Infant and Mother-Infant Dyads: Attunement or Something More?

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Author Note

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Data Availability Statement

The data that support the findings of this study are available from Brenda L. Volling (volling@umich.edu) upon reasonable request.

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Abstract

Father-infant and mother-infant (one-year-olds) adrenocortical attunement was explored during the Strange Situation Procedure (SSP) among 125 father-infant and 141 mother-infant dyads. Cortisol was assessed at baseline (T1), 20 (T2) and 40 minutes (T3) after the first parent-infant separation. Initial correlations indicated significant associations between father-infant and mother-infant cortisol at each time. Cortisol interdependence was further explored using Actor-Partner Interdependence Models. There was no evidence supporting cortisol interdependence based on within-time residual correlations between parent-infant cortisol, once stability and cross-lagged paths were controlled. Infant cortisol at T2 predicted T3 cortisol for fathers and mothers resulting in a series of follow-up exploratory analyses to examine mediating processes which revealed that infant distress during the SSP predicted infant T2 cortisol, which, in turn, predicted infant negativity during the 15-minute mother-infant teaching task that followed the SSP. Among father-infant dyads, infant T2 cortisol predicted infant negativity during father-infant interaction, with infants expressing more negativity having less sensitive fathers. Findings provide little support of parent-infant adrenocortical attunement across either father-infant or mother-infant dyads during the SSP, but preliminary evidence indicates infant distress as a potential mediator. Future research may want to focus on affective and behavioral processes that underlie the concept of parent-infant adrenocortical attunement.

Keywords: Adrenocortical attunement, father-infant relationship, mother-infant relationship, cortisol, stress reactivity

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Dysregulation of the hypothalamus-pituitary-adrenal axis (HPA-axis) early in life has implications for future psychopathology (Gunnar & Donzella, 2002), and parents may play a role early on in helping infants regulate their HPA-axis response to stress (Laurent et al., 2012; Luijk et al., 2010). When parents begin to recognize their infant's signals, parent-infant behavioral synchrony develops and is driven by the physiology behind bond formation (Feldman, 2007, 2015). The physiological synchrony between parents and their infants is often called attunement, referring to diurnal or stress-reactive cortisol relations between parents and their infants. Cortisol attunement has been studied mostly among mother-infant dyads (Atkinson et al., 2013; Khoury et al., 2016; Van Bakel & Riksen-Walraven, 2004), but the current study will advance research on adrenocortical attunement by focusing on both father-infant and mother-infant dyads and assessing attunement using Actor-Partner-Interdependence Models (APIM), in which we focus on cortisol interdependence (within-time residual correlations between parent-infant cortisol), in addition to the actor and partner paths of the model in an effort to disambiguate different processes that may underlie the concept of attunement. When we refer to adrenocortical attunement, we mean the investigation of all of the potential significant paths between parents and infants, including the cross-lagged, stability, and within-time residual correlations. If adrenocortical attunement is indeed a reflection of the biological and behavioral synchrony between parents and infants across stressful situations, then attunement should describe interdependence across both father-infant and mother-infant relationships. We know from other research on differences in father-infant versus mother-infant interaction that fathers and mothers play differently with their infants (Feldman et al., 2010; St. George et al., 2018), and mothers still spend more time in childcare compared to fathers (LaFlamme et al., 2002; Sayer et al., 2004). Thus, adrenocortical attunement during stressful situations may look different in father-infant dyads compared to mother-infants dyads. The primary goal of this study was to evaluate these potential differences or similarities in adrenocortical attunement between father-infant and mother-infant dyads.

Individual Differences in Dyadic HPA-Axis Attunement

The early emergence of bio-behavioral synchrony between the regulatory rhythms of infants and their caregivers is theorized to be a critical component of adaptive socio-emotional

development (Feldman, 2012a; Rayson et al., 2017). Well before the development of verbal language and higher-order social capacities, infants learn how to regulate their distress and behavior in the context of early parent-infant interactions. This implicit learning process has its roots in the precociously emerging oscillatory and rhythmic coupling of physiological and biological systems for stress, attention, and emotion regulation between the two interactive partners (Feldman, 2006). More specifically, the co-regulation of the main neuroendocrine system for stress regulation – the HPA-axis – is not only a reflection of how the dyad typically responds behaviorally to stressful events, but is also a process involved in adaptive infant socio-emotional development (Muller et al., 2015). For example, Hibel and colleagues (2015) found that more emotionally reactive infants demonstrated less attuned cortisol responses with their mothers during a series of infant-directed stressors. Nofech-Mozes and colleagues (2019) found that infant attachment was related to cortisol attunement during the Strange Situation Procedure (SSP). Infants classified as disorganized had greater cortisol difference scores with their mothers compared to organized infants (Nofech-Mozes et al., 2019). These studies demonstrate how mother-infant cortisol attunement is associated with infant social-emotional behaviors such as infant emotional reactivity and attachment, which may further impact infant social-emotional development down the line. Thus, parent-infant cortisol attunement warrants investigation because of its potential ties to the social-emotional outcomes for infants as they grow.

Nonetheless, wide differences exist in how researchers investigate adrenocortical attunement (Bernard et al., 2017; Davis et al., 2018). Such variations may reflect different theoretical and procedural definitions of adrenocortical attunement, and the manner in which parent and infant cortisol is statistically analyzed, all of which may give rise to different findings and interpretations (Bernard et al., 2017; Nofech-Mozes et al., 2019). Further, cortisol attunement has also been studied looking at both diurnal and stress-reactive processes (Thompson & Trevathan, 2008; Van Bakel & Risken-Walraven, 2004). In review, terms such as attunement, synchrony, coordination, and co-regulation are often used interchangeably to describe how parent and infant cortisol are related either diurnally or via stress reactivity. Further, research designs differ dramatically based on the procedures used, whether cortisol is collected as part of a diurnal cycle or a measure of stress-reactivity in a laboratory, how many cortisol assessments are collected, when they are collected, the age of the infants examined, and the statistical analyses conducted (Crockett et al., 2013; Laurent et al., 2011). For example,

Crockett and colleagues (2013) used the term synchrony to describe how mother and infant cortisol converged or diverged systematically (assessed during the Still-Face Procedure) using a mixed model ANOVA and calculated the absolute difference between mother and infant cortisol as the dependent variable (Crockett et al., 2013). Laurent and colleagues (2011) used the term attunement to describe when partners' stress responses "occur in parallel" across the SSP and utilized hierarchical linear modeling by entering mothers' cortisol as the time-varying covariate predicting infant cortisol. Thompson and Trevathan (2008) preferred to describe cortisol coordination as significant positive correlations between mothers' and their 18-month-old infants' cortisol during a brief mother-infant separation.

Exploring the Basis of Cortisol Attunement

In the current report, we observed parents (fathers and mothers) and their infants during separate laboratory visits involving the SSP and collected saliva for cortisol assays from both participants at three times over the course of the visit to examine adrenocortical interdependence. We prefer the term interdependence to describe how parent and infant cortisol are related within the same time point and use the dyadic Actor-Partner-Interdependence Model (APIM) to model cross-time stability within the same individual (Actor effects) for infants and parents, the prediction of infants' cortisol from parents' cortisol at an earlier time (Partner effect), the prediction of parents' cortisol from infants' cortisol at an earlier time (Partner effect), and the within-time relations between parent and infant cortisol (Interdependence effects) once actor and partner effects are controlled. The APIM was developed to address the issue that dyadic data are nonindependent; the correlated residual term provides a measure of interdependence within the dyad (hence the I in APIM; see Kenny et al., 2006; Gonzalez & Griffin, 2001). The other terms in the APIM (the A and the P) refer to cross-time stability (Actor) and cross-person (Partner) effects that are separate concepts from the synchronous association of the residuals. These three terms decompose the covariance structure to permit clearer inferences about dyadic processes that may underlie relations uncovered in APIM. For example, what may appear at first as a dyadic pattern of attunement could arise from stability within the same individuals (Actor effects) and a baseline correlation across individuals (i.e., infants who are above the mean remain above the mean, parents who are above the mean remain above the mean, and infants above the mean tend to have parents above the mean), without any subsequent within-time association involving interdependence over time.

Adrenocortical attunement for us, then, describes a model where the within-time residual correlations, as well as the actor and partner effects are all examined for significance to reveal the different dyadic processes of an APIM with the goal of further refining what is at play in attunement processes. Previous studies have utilized the APIM to investigative associations between mother and infant cortisol. For instance, Bernard and colleagues (2017) found that mothers' baseline cortisol predicted one-year-old infants' cortisol 20- and 40-minutes post-arm restraint, and Nofech-Mozes and colleagues (2019) found that both mother and infant cortisol predicted subsequent partner cortisol. Both of these studies, however, only examined the partner and actor effects for mother-infant dyads but not the within-time cortisol interdependencies between mothers and infants, which is the third important component of the APIM. The ability to investigate within-time interdependencies between parent and infant cortisol while controlling for partner and actor effects across time is an advantage of utilizing APIM. A model representing the conceptual APIM structure for testing cortisol interdependence and attunement can be found in Figure 1.

Father-Infant Cortisol Attunement

Whereas most research to date has focused on mother-infant adrenocortical attunement, few studies have considered cortisol attunement between infants and their fathers, even though infants form attachment relationships with their fathers (Kuo et al., 2019). Prior research has demonstrated oxytocin synchrony between fathers and infants, suggesting transmission in the neurobiology of affiliation (Feldman, 2012b; Feldman, Gordon, & Zagoory-Sharon, 2010). The quality of the infant-father attachment relationship and certain fathering behaviors are also linked to infants' cortisol responses to stress. For instance, Mills-Koonce and colleagues (2011) investigated the association between observed paternal sensitivity and paternal negativity with infant cortisol responses to increasingly emotionally arousing tasks (e.g., toy removal, arm restraint) when infants were seven and 24 months. Father negativity at seven-months was positively linked to infants' increased cortisol levels 20-minutes after the tasks, and father negativity at 24-months was also positively associated with infants' baseline cortisol levels at 24-months (Mills-Koonce et al., 2011). These findings suggested that the quality of father-infant interactions during potentially stressful experiences played a role in how infants' HPA-axis responded to stress. Although Mills-Koonce and colleagues (2011) did not test father-infant cortisol attunement, parenting behavior and the ability to soothe and help an infant regulate their

distress may very well be a process underlying cortisol attunement, rather than expecting a direct link between the HPA-axes of infant and parent.

To our knowledge, only two studies to date have investigated cortisol attunement comparing father-child and mother-child dyads. Saxbe and colleagues (2016) found significant father-child, but not mother-child, cortisol correlations, albeit among kindergarten-aged children and not infants, during a series of emotion regulation tasks. In the study by Stenius and colleagues (2008) on parent-infant attunement, correlations revealed that mother-infant morning, afternoon, and evening diurnal cortisol levels were strongly correlated, whereas only afternoon and evening cortisol levels were correlated between fathers and infants, albeit weaker than in mother-infant dyads. These findings suggest that the strength of interdependent cortisol associations may be weaker in father-infant dyads but stronger between fathers and their kindergarten-aged children compared to mothers and their children. Further research is needed to replicate these findings, and additional analyses in the APIM framework could tease apart the stability, cross-person and interdependence features of these associations because it is not clear why parent-child cortisol interdependence or attunement to stress or attunement or interdependence in diurnal levels should differ across father-child and mother-child dyads. Further, without comparisons across father-child and mother-child dyads, it is not clear what accounts for variation in cortisol interdependence and attunement. Kuo and colleagues (2019) recently found that when infants had a secure attachment to their fathers, but an insecure attachment to their mothers, they exhibited a hyperresponsive trajectory of cortisol across the SSP with higher levels of cortisol at baseline that declined over time compared to infants with secure attachments to both parents, a secure attachment to mother but insecure to father, or when both attachments were insecure; all of whom exhibited a more typical cortisol response to stress reflecting an increase (reactivity) and then decrease (recovery). Fathers and mothers often interact differently with their infants, with fathers engaged in more physically stimulating play and mothers, more object-mediated and verbal play (Feldman, Gordon, Schneiderman, et al., 2010 for review see St. George et al., 2018). Further, mothers are still responsible for more childcare than are fathers, in general, and therefore, spend more time with their infants, so one might expect stronger interdependent relations between mothers' and infants' cortisol than between fathers and infants (Laflamme et al., 2002; Sayer et al., 2004). Collectively, these findings suggest that the quality of parenting and the different relationships infants have with

their fathers and mothers may modify infants' cortisol responses to stressful experiences, and hence, patterns of cortisol interdependence and attunement within parent-infant dyads.

The Current Study

The current study assessed both father-infant and mother-infant cortisol interdependence and attunement with one-year-old infants in response to the SSP using the APIM model, a widely used model for the analysis of dyadic and relationship constructs, including studies on mother-infant interactions (Boeve et al., 2019) and in particular, mother-infant cortisol attunement (Bernard et al., 2017; Nofech-Mozes et al., 2019). As wide variations exist in the conceptual and operational definitions of dyadic adrenocortical attunement in the mother-infant relationship (Bernard et al., 2017), as well as the type of statistical modeling utilized to measure cortisol interdependence and attunement (Bernard et al., 2017; Davis et al., 2018; Nofech-Mozes et al., 2019), we utilized similar modeling strategies here to explore both father-infant and mother-infant adrenocortical interdependence and attunement to determine whether there were similarities or differences across mother-infant and father-infant dyads. To do so, we first conducted all modeling using data from mother-infant dyads given that most research on cortisol attunement to date has been conducted with mothers and infants, and then utilized data from father-infant dyads to replicate these findings. Parent-infant attunement may be based on several dyadic processes that underlie the APIM model including significant stability (Actor effects) and cross-lagged paths (Partner effects) for parent and infant cortisol across the three times of measurement, as well as significant within-time residual correlations between parents and infants (Interdependence).

Methods

Participants

Participants were mothers, fathers, and their secondborn 12-month-old infants participating in a longitudinal investigation looking at family relationship change after the birth of a second child with five times of assessment; the last trimester of the mothers' pregnancy with the second born, and 1, 4, 8 and 12 months following the birth. Mothers were the biological mothers of both the firstborn and secondborn, and fathers were required to be the biological fathers of the secondborns (98% were also the biological father of the firstborn). Mothers and fathers were also required to be cohabiting (99% were married). The larger investigation recruited 241 two-parent families in which mothers were expecting their second child (see

author citation blinded for review for full details of the recruitment, procedures, and measurement design). The current report, however, focuses on the Hormones, Behavior and Parent-Infant Relationships Sub-study (HBPIRS) conducted with mothers, fathers, and infants at 12 months, during counterbalanced laboratory visits in which the SSP was conducted. Recruited families were primarily European American (86.3% of fathers, 85.9% of mothers), had at least a bachelor's degree (79.2% of fathers and 83.9% of mothers), and the median family income was $60,000-$99,999. Couples had been married for an average of 5.77 years (SD = 2.74). Mothers worked, on average, 29.88 hours (SD = 15.32) per week and fathers worked, on average, 45.50 hours (SD = 12.30) per week.

Of the initial 241 families, 203 families remained at the 12-month timepoint, and of those 203, 180 parents and their secondborn infants (99 boys) consented to participate in HBPIRS. Data for the current analyses included participants (141 mother-infant dyads and 125 father-infant dyads) who each (both parent and infant) had at least one cortisol sample across the three measurement occasions (described below). Cortisol samples were missing due to low saliva sample volume or difficulty in obtaining a sample from infants. Families that remained at the 12-month timepoint had higher incomes, $\chi2$ (3) = 13.94, p < .01, and both mothers and fathers had higher education, , $\chi2$ (2) = 7.90, p < .05, , $\chi2$ (3) = 10.82, p < .05, compared to the initial 241 families. Families that remained at 12-months did not differ on parents' years of marriage, mothers' or fathers' age, or mothers' or fathers' race/ethnicity compared to the initial 241 families.

Previous reports from this study have also focused on hormonal variation across parents and infants in the 12-month laboratory visit, including patterns of infants' cortisol responses based on the security of their attachment relationships with both fathers and mothers (blinded for review), individual variation in fathers' testosterone across the visit and associations with fathering behaviors during father-infant interactions (blinded for review), and parents testosterone reactivity during parent-infant interaction related to parents' adult attachment orientation (blinded for review). The current report builds on these previous studies by investigating cortisol interdependence and attunement between mothers and infants, and then attempting to replicate these findings with fathers and infants. The study was approved by the Institutional Review Board of the University of blinded for review (IRB-MED).

Measures

Study Procedures

The HBPIRS was designed to investigate parent and infant hormones, parent-infant interactions, and infant attachment security at 12-months. The laboratory visits for mother-infant and father-infant sessions were identical, but counterbalanced and separated by one month (12 and 13 months); a recommendation based on prior research to reduce the likelihood of infant memory for the procedures and to reduce emotional contagion (Braungart-Rieker et al., 1999; Volling & Belsky, 1992). The laboratory procedures included an interview to collect infant health and temperament data, the SSP, a brief break of about 5 minutes, and a 15-minute parent-infant teaching task. Saliva was collected from both parents and infants at three times during the visit (1) after completion of the interview and right before the SSP (Time 1, baseline); (2) approximately 20 minutes after the first parent-infant separation of the SSP (episode 4), which often coincided with the completion of the SSP (Time 2); and (3) 20 minutes later or 40 minutes after the first parent-infant separation (Time 3), which typically occurred after the 15-minute teaching task. These times were chosen because the cortisol response to stress usually peaks between 15-40 minutes following a stressor for both infants and adults (Goldberg et al., 2003; Kemeny, 2003), so we could assess a cortisol reactivity (Time 1 to Time 2) and recovery response (Time 2 to Time 3). Saliva samples were collected from infants using two Sorbette Swabs (Sarstedt) in the mouth until the swabs were saturated, around 60-90 seconds. Parents' saliva was stimulated by chewing Trident Original sugarless gum, which does not affect cortisol (Schultheiss, 2013), and they then provided 10 mL of saliva through passive drool into 50 mL polypropylene tubes (United Lab Plastics).

Strange Situation Procedure

The SSP (Ainsworth et al., 1978) is a standard laboratory procedure used to assess both father-infant and mother-infant attachment security around one year of life, but parent-infant separations, which are part of the SSP, are also reliable infant stressors that have been used to measure both mother and infant cortisol (Goldberg et al., 2003; Laurent et al., 2012; Luijk et al., 2010). The SSP includes seven, 3-minute episodes involving a series of separations and reunions between the parent and infant.

Infant Distress. Infant distress was scored during the two separation episodes (episode 4 and 7) of the SSP and episode 6 in which only the stranger and infant were together in the room. Infant distress was scored on a 5-point scale, 1 = no evidence of distress when parent was absent

to 5 = immediate or nearly immediate full distress and no indications that child would settle. Infant distress was coded by two independent coders, and the average kappa across parents and episodes was .96. Infant distress scores were averaged across the three episodes. Infant distress did not significantly differ between mother-infant or father-infant dyads, $t(116) = 1.34$, $p = .183$.

Teaching Task

The 15-minute teaching task for each visit consisted of parents teaching their infants to complete thee different tasks involving three different toys, presented in separate boxes that included a set of instructions (i.e., hitting all the xylophone keys with a mallet; working all the buttons on a Sesame Street activity box; hitting all shapes on the back of a turtle with the hammer; see (Vondra et al., 1995). Each task was to be 5 minutes in length, and parents were told all tasks were beyond the ability of a 12-month-old to do alone so they should try and teach their infants how to complete them. Each five-minute episode was coded separately by a trained coder (five total coders) for both infant and parent behaviors using a coding system adapted from the NICHD Study of Early Child Care Research Network (2000). Episodes were scored from 1 = not at all characteristic to 7 = very characteristic. Infant negative mood assessed the extent to which infants cried and fussed or otherwise expressed discontentment (average ICC = .95). Parental sensitivity assessed how observant and responsive parents were to infants' social cues and expressions; highly sensitive parents are child-centered and aware of the infant's needs, interests, and capabilities (average ICC = .86).

Salivary Cortisol

All saliva samples were stored at -80 degrees Celsius prior to assay. Infant saliva samples were separated from hydrocellulose absorbent swabs and parent samples from polypropylene tubes upon thawing and expressed into 2-mL cryogenic vials until being centrifuged for assay. Infant, mother, and father samples were assayed in different batches on the same day. Samples were assayed using highly sensitive enzyme immunoassay (Salimetrics, State College, PA). Duplicate samples were assayed and the average value was included in analyses. Samples with low volume were assayed in single wells. The intra-assay and inter-assay coefficients of variation were on average, 11.0% and 18.78%, respectively. The test had a range of sensitivity from 0.007 to 3.0 mg/dl, so any cortisol values that were greater than 3.0 were removed from the analyses. Three infants in the mother-infant SSP and five infants in the father-infant SSP had cortisol values greater than 3.0, and thus these values were removed from the analyses. Cortisol

values were log-transformed due to positive skew typical with human cortisol data. A 2 (parent) x 3 (time) x 2 (infant gender) linear mixed model indicated that there were no significant time, gender, or parent effects, or significant interactions. Thus, infant cortisol levels did not differ during the SSP if infants were with fathers compared to mothers or by infant gender. Parent-infant dyads came to the laboratory for the SSP at different times during the day in an effort to accommodate parents' schedules and to complete data collection for the large number of mother and father visits (approx. 400) for this study. Thus, the time of day based on when the Time 1 cortisol sample was collected was controlled for in the partial correlations, APIM, and post-hoc analyses.

Data Analysis Plan and Preliminary Findings

As a first step, we conducted descriptive statistics and partial correlations between cortisol values for parents and infants across the three times of measurement (see Table 1). Examining within-time intercorrelations between parent and infant cortisol is one means used in previous research to assess attunement (Middlemiss et al., 2012; Thompson & Trevathan, 2008). Mothers' and infants' cortisol levels were significantly correlated at all three time points, as were fathers' and infants' cortisol, providing preliminary evidence for parent and infant cortisol attunement. But, such correlational analyses do not take into consideration stability in individual differences across time (actor effects) or the cross-lagged associations between parent and infant cortisol across time (partner effects) which once controlled in analyses, may alter within-time interrelations.

Next, mean level change in cortisol across time for the entire sample of parents and infants was examined using four Linear Mixed Models (LMMs); one for each parent and one for infants during mother visits and father visits-, using R 3.6.1 in which cortisol levels were the outcome variables and the three times of measurement were treated as categorical predictors; random intercepts for each child were included. LMM analyses revealed that mothers' cortisol decreased significantly from T1 to T2, β = -.35, 95% CI = [-.46, -.24], from T1 to T3, β = -.60, 95% CI = [-.71, -.48], and from T2 to T3, β = -.24, 95% CI = [-.36, -.13]. Infants' cortisol levels were not significantly different across the three time points during the mother-infant SSP. In the father-infant SSP, fathers significantly declined in cortisol from T1 to T2, β = -.42, 95% CI = [-.54, -.31], and from T1 to T3, β = -.53, 95% CI = [-.65, -.41], but not from T2 to T3, β = -.11, 95% CI = [-.23, .01]. Infants' cortisol was not significantly different from T1 to T2, β = .14, 95%

CI = [-.05, .34], and T1 to T3, β = -.13, 95% CI = [-.33, .06], but significantly decreased from T2 to T3, β = -.28, 95% CI = [-.47, -.08]. Figures S1 and S2 in supplemental materials also show the mean-level change in mother and infant cortisol, and father and infant cortisol, respectively. Given the different mean trajectories, cortisol is clearly not changing similarly across time for parents and infants.

The main aim of this investigation focused on adrenocortical interdependence between mother-infant and father-infant dyads using APIM. We conducted the APIMs in MPlus 8.4 using full information maximum likelihood estimation to analyze mother-infant and father-infant cortisol interdependence and attunement effects. Because mother-infant dyads and father-infant dyads were observed in the SSP during separate laboratory visits spaced approximately a month apart, APIM models were run separately for mother and father sessions. The cortisol correlation within dyads at T1 was estimated, as well as the residual correlations within-dyads at T2 and T3, which constitutes the interdependence between parent and infant cortisol.

APIM analyses were used to investigate relations between parent and infant cortisol examining stability across time for each individual (Actor), cross-lagged associations across individuals (Partner) and interdependence within-time. Unlike simple correlations, APIM allows us to examine the interdependence (Gonzalez & Griffin, 1997; Kenny et al., 2006) between father-infant and mother-infant cortisol values, while taking into consideration both the stability and cross-lagged paths. This is a slightly different approach than the APIM models reported by others (e.g., Bernard et al., 2017; Nofech-Mozes et al., 2019) who did not include estimates of interdependence (the correlated residuals) between parents and infants at the times of cortisol assessment. Because APIM is inherently a modeling strategy designed to investigate dyadic patterns which may be the basis of attunement, it is important to report and examine all three components: the stability (actor), the cross-lagged (partner) paths, and the interdependence (correlated residuals) associations. Different dyadic patterns can emerge which require different interpretations, and we considered two of the most common here (see Fitzpatrick et al., 2016). The dyadic-oriented pattern indicates that the stability (actor effects) of infant cortisol across T1, T2 and T3 is the same as the cross-lagged relations (partner effects of parent on infant cortisol). The opposite can also be true such that stability in parent cortisol across time is equivalent to the effect of infant cortisol predicting parent cortisol over time (the interdependence term is estimated as well). If even one of the stability or cross-lagged paths are not significant in the

APIM, then we can no longer speak of a dyadic-oriented pattern, and hence, attunement. A second pattern referred to as the actor-only pattern, is one in which an individual's cortisol at one time has a significant effect on their cortisol at a later time (stability), but there are no cross-lagged partner effects (the interdependence term is estimated as well). If only the stability paths are significant (actor-only), there is only evidence that the individual's cortisol (either parent or infant) predicts their own cortisol across time, but is not affected by or affects the other member of the dyad. As a reminder, all models were run first using mother-infant cortisol values before attempting to replicate the findings using father-infant cortisol values.

Results

Adrenocortical Interdependence and Attunement in Mother-Infant and Father-Infant Dyads

Using the APIM, we examined actor and partner effects, as well as the interdependence between mother and infant cortisol (see Figure 2), $\chi^2 (8) = 39.78$, p < .001, CFI = .88, TLI = .69, RMESA = .17. All the stability coefficients (actor effects) were significant for mothers and infants over time. With respect to partner effects, or the effect of one individual's cortisol to predict their partner's cortisol at a subsequent time, the only significant partner effect was infant cortisol at T2 predicting mother cortisol at T3, with greater infant cortisol after the SSP predicting higher levels of mothers' cortisol at T3, 20 minutes later. Although mothers' and infants' cortisol were initially significantly correlated at both T2 and T3 (see Table 1), the residual correlations between mother and infant cortisol at T2 and T3 in the APIM were no longer significant, suggesting that there was no within-time interdependence between mother and infant cortisol once actor and partner effects were included.

Similar findings were uncovered for father-infant dyads in the APIM, $\chi^2 (84) = 20.10$, p = .010, CFI = .96, TLI = .90, RMESA = .11. As can be seen in Figure 2, the APIM results show significant actor effects for both fathers' and infants' cortisol, but only a significant partner effect for infant cortisol at T2 predicting father cortisol at T3. As was the case with mother-infant dyads, higher infant cortisol at T2 significantly predicted higher father cortisol at T3. Once again, even though correlations between father and infant cortisol were significant (see Table1) the residual correlations in the APIM model reflecting interdependence were no longer significant.

Exploratory Post-Hoc Analyses

Once the APIM results revealed limited support for cortisol interdependence and attunement across mother-infant and father-infant dyads, we developed a series of post-hoc exploratory models that focused specifically on the partner effect found in both the mother and father APIM models with infant cortisol at T2, measured directly after the SSP, predicting higher levels of parent cortisol at T3, nearly 20 minutes later after the parent-infant teaching task, with the understanding that the association between infant physiology and parent physiology is more than likely mediated through other affective (i.e., infant distress aroused by the SSP that may carry over into the subsequent parent-infant interaction) and behavioral processes (i.e., the degree of parental sensitivity used during parent-infant interaction to help alleviate and reduce the infant's affective arousal). We describe each of these post-hoc exploratory models below but note here, that all models were once again conducted first with mothers and infants before attempting to replicate results with fathers. All model fit indices for these exploratory models are shown in Table S1 in supplemental materials.

Model testing for mothers. The first follow-up model tested an infant distress model in which the infants' distress in the SSP (an observable indicator of infant stress to parents) would be a common factor predicting both infant and parent cortisol at both T2 and T3 (see Figure 3). Here, infant distress in the SSP did indeed predict greater infant cortisol at T2 following the SSP, but did not predict infant cortisol at T3 or mother cortisol at either T2 or T3. The second model, the infant affective spillover model (see Figure 4) shows that infant distress in the SSP predicted greater levels of infant cortisol at T2 as above, which, in turn, now predicted more infant negativity during the teaching task that followed (i.e., spillover), yet infant negativity did not predict mothers' or infants' cortisol at T3. The third model, the parent stress model, was similar to the infant affective spillover model, but infant negativity during parent-infant interaction was replaced with parental sensitivity observed during the teaching task, hypothesizing that stressed parents after the SSP would be less sensitive in their interactions, particularly in response to infant negativity, which may account for higher infant cortisol at T3 and also be related to their T3 cortisol. Results are shown in Figure 5, and show that maternal sensitivity is not a mediator between infant and mother cortisol from T2 to T3. Finally, the parent-infant coregulation model is shown in Figure 6, in which we included both observed infant negativity and parental sensitivity to reflect the relations between infant and parent during parent-infant interaction. Results showed once again that infant distress in the SSP predicted infant cortisol at T2, which

significantly predicted infant negative affect during mother-infant interaction in the teaching task, but infant negative affectivity was not significantly correlated with mothers' sensitivity. There were no further significant paths supporting the behavioral coregulation of mother-infant interaction as a mediator between infant and mother physiology.

Model testing for fathers. In the infant distress model, infant distress in SSP did not significantly predict infant or father cortisol at either T2 or T3 (see Figure 3). In the infant affective spillover model shown in Figure 4, infant distress in SSP did not predict infant or father cortisol at T2 or T3. Infant cortisol at T2 did predict less infant negativity in the teaching task that followed the SSP, the opposite of what would be expected if distress positively spilled over into father-infant interaction, and in contrast to results found for mother-infant dyads. Infant negativity during the teaching task interaction did not predict fathers' or infants' cortisol at T3, providing no support for infant negativity as the mediating path between infant T2 cortisol and father T3 cortisol (the significant partner effect from the earlier APIM). In the parent stress model, there was no evidence of paternal sensitivity in the teaching task as a mediator between infant T2 cortisol and father T3 cortisol (see Figure 5). Finally, the parent-infant coregulation model is shown in Figure 6. As with the earlier model, infant cortisol at T2 significantly predicted less infant negativity during father-infant interaction, which, in turn, was negatively related to paternal sensitivity. Even though fathers were less likely to respond sensitively to infant negativity, there was no direct prediction from either infant negativity or paternal sensitivity predicting infant or father cortisol at T3 that would explain the infant T2 to father T3 partner effect.

Discussion

Our study is the first exploratory analysis of cortisol interdependence and attunement between both father-infant and mother-infant dyads during the laboratory SSP with one-year-old infants. We chose to include fathers in the current research program because we know that fathers have the capacity for biobehavioral attunement with their infants (Feldman 2012b; Feldman et al., 2010) and infants form secure attachments to their fathers (Volling & Belsky, 1992). However, to date, adrenocortical interdependence and attunement during stressful encounters between infants and their fathers has not been investigated. Preliminary analyses indicated significant partial correlations between infant and parent cortisol at each time for both father-infant and mother-infant dyads. However, the APIM analyses revealed that only parent

and infant cortisol at time 1 (baseline – prior to SSP) was statistically significantly correlated (i.e., cortisol interdependence) in mother-infant and father-infant dyads, and there was no evidence of parent-infant cortisol interdependence at T2 and T3 once controlling for the actor and partner effects of infant and parent cortisol across time. Similarly, mean-levels of parent and infant cortisol changed differently, not similarly, over the lab visit, which, one would expect to be the case if HPA-axis functioning for parents and infants was attuned. Mean levels of cortisol showed that both fathers and mothers demonstrated higher cortisol levels upon arrival to the laboratory, and these levels gradually declined over time, with no indication of a cortisol stress response to seeing their infants' distress during the separations of the SSP. Infants displayed different patterns from their parents' hyperresponsive pattern. Infants were more likely to display a cortisol reactivity-recovery response with an increase in cortisol from baseline (T1) to T2 following the SSP, and then a decline from T2 to T3 representing a return to baseline cortisol levels. These mean level patterns suggest that parents and infants do not respond the same physiologically to the SSP visit. It should be noted that our preliminary findings of significant within-time correlations and different mean-level change were replicated across father-infant and mother-infant dyads, as were the results of significant, predominantly actor effects, reflecting stability in individual differences in parent and infant cortisol, in the APIM models. Such consistency in findings across mother-infant and father-infant dyads provides strong evidence that the findings reported here are not unique to one parent-infant dyad, but appear to be similar across both mother-infant and father-infant relationships.

As part of the replicated results, we also found that infants' cortisol at T2 (20-minutes following parent-infant separation in the SSP) significantly predicted both mothers' and fathers' cortisol at T3 (40-minutes following parent-infant separation in the SSP). Significant crossed-lagged paths have been found in research on mother-infant cortisol attunement (Bernard et al., 2017; Nofech-Mozes et al., 2019), albeit with different patterns. Bernard and colleagues (2017) found that mothers' time 1 (baseline – prior to arm restraint task) cortisol predicted infants' (approximately one year old) cortisol 20-minutes follow the arm restraint task, and mothers' time 2 cortisol predicted infants' time 3 cortisol 40-minutes following the arm restraint task. Nofech-Mozes and colleagues (2019) found that a mother's previous cortisol value predicted her infant's subsequent cortisol value, and an infant's previous cortisol value predicted a mother's subsequent cortisol value in response to the SSP (all three samples were collected at similar

times as those in our study). However, Nofech-Mozes and colleagues (2019) did not indicate which of the two cross-lagged paths (between time 1 and 2 or between time 2 and 3) were related between mothers and their infants. Nonetheless, both the study by Nofech-Mozes and colleagues (2019) and Bernard and colleagues (2017) describe these significant processes as indicating mother-infant adrenocortical attunement. Unlike our study, however, these two studies did not include a within-time cortisol interdependence effect, once cross-lagged, partner paths and stability, actor paths were controlled. We argue that these interdependent paths are an essential part of parent-infant cortisol attunement because they explain the remining cortisol associations between parents and infants after accounting for infants' and parents' own cortisol as well as the effects of each individuals' cortisol on the partners' subsequent cortisol. Also, these interdependent paths are associations between parent and infant cortisol at the same time rather than cross-lagged associations meaning that they are measuring associations in cortisol output from both parents and infant simultaneously or in parallel.

Uncovering the Infant T2 to Parent T3 Effect

The finding that infants' T2 cortisol significantly predicted parents' T3 cortisol warranted further investigation as to what might be the mediating mechanism(s) linking the adrenocortical response of an infant to a laboratory stressor with the subsequent adrenocortical response of an adult caregiver 20 minutes later. There appeared to be some support for the *infant distress* and *infant affective spillover* models. As we suspected, observable infant distress during the SSP was associated with infants' T2 cortisol collected immediately following the SSP, and infant T2 cortisol then predicted the infants' negativity during mother-infant interaction in the teaching task that followed. However, infant distress did not predict infants' or mothers' T3 cortisol, nor was there any relation between infant negativity or mother's behavioral sensitivity during mother-infant interaction and mothers' T3 cortisol, leaving the initial partner effect between infant and mother cortisol across T2 and T3 unexplained. Thus, it appears that infants' observable distress only predicted infant's cortisol reactivity (20-minutes post-stressor) but not recovery (40-minutes post-stressor). Egliston and colleagues (2007) have pointed out the less than consistent findings between infant behavioral distress and cortisol reactivity and recovery during inoculations, in which both positive associations, as well as no associations, have been found (e.g., Lewis & Thomas, 1990; Ramsay & Lewis, 2003).

In contrast to the mother-infant dyads, we did not find that infants' distress during the SSP predicted their T2 or T3 cortisol with fathers, yet infant T2 cortisol after the SSP was associated with their negativity during father-infant teaching interactions, but inversely, such that more infant T2 cortisol was related to less infant negativity. Thus, infant T2 cortisol reactivity was linked to their negative affect in the teaching task in different ways for father-infant and mother-infant dyads. One explanation may be that fathers, witnessing their infants' distress during the separations in the SSP, react differently themselves to their infants' negative affect during both the SSP and while interacting with their infants given that fathers have been observed in some cases to be more intrusive and less sensitive in teaching tasks than mothers (Volling et al., 2002). Indeed fathers were less sensitive in response to infant negative affect in the current study, but neither infant negativity nor lower paternal sensitivity predicted increases in T3 cortisol for infants or fathers, and therefore, could not account for the T2 to T3 partner effect.

We also considered whether observing their infant's distress during the SSP might act as a stressor for parents that might account for the T3 increase in cortisol for both fathers and mothers. But, we found no support from the *infant distress model* supporting this assumption, or the *parent stress model*, as there was no relation between infant distress during the SSP and either parents' T2 or T3 cortisol levels, or their insensitive behaviors with their infants. Similar to the different patterns of mean-level change found for infants and their parents, perhaps the SSP should not be viewed as an equivalent stressor for infants and adult caregivers, leading one to wonder whether one should anticipate adrenocortical attunement across adults and infants in either father-infant or mother-infant dyads when using this laboratory paradigm. Because fathers and mothers had higher baseline cortisol values that declined post-separation, parents may be experiencing an anticipatory stress response reflective of their uneasiness with the novelty of a visit to the university laboratory that declines as they become acclimated to the environment (Gunnar & Vazquez, 2006). Similar to our results, Hibel and colleagues (2015) also found that mothers' cortisol values declined from baseline assessment during another infant-focused stressor – the Laboratory Temperament Assessment Battery (Lab-TAB).

In none of the *post-hoc* models examined did we find infant distress in the SSP, or infant negativity and parental sensitivity during teaching task interactions, related to mothers' or fathers' cortisol reactivity (T2) or recovery (T3). Thus, it appears that parents and infants are not

experiencing the SSP in the same way, so investigating parent-infant cortisol interdependence and/or attunement may not be warranted in this type of procedure. The SSP was designed and intended to assess the quality of infant-parent attachment security, but because the SSP includes separations between parents and infants, which are known infant stressors, researchers have used the SSP to study parent-infant cortisol attunement without considering that adult caregivers and infants are indeed responding differently to this infant-directed stressor.

Even though we did find that partial correlations between infants' and parents' cortisol were significant at all three times for both father-infant and mother-infant dyads, the within-time cortisol interdependencies at T2 and T3 were no longer significant in the APIM. Our findings provide more support for an *actor-only* model where infants' and parents' cortisol at a prior time is the independent variable that predicts their own subsequent cortisol (dependent variable) versus a *dyadic-oriented* (actor and partner paths are significant) or the rare *partner-only* APIM patterns, where parents' and infants' cortisol predicts subsequent partner cortisol only (Fitzpatrick et al., 2016; Kenny & Ledermann, 2010). Thus, we do not find strong evidence of dyadic adrenocortical processes in our APIM models between parents and infants and more evidence of stability in individual parents' and infants' own cortisol output over time. This is in line with Fitzpatrick and colleagues (2016) who argue that a dyadic-oriented pattern in an APIM is not indicated when at least one of the stability or cross-lagged paths is insignificant. However, we do find evidence that both mother-infant and father-infant cortisol are linked in some way when infant cortisol following separation from a parent predicts subsequent parent cortisol levels, but it is unclear what predicts this association.

Limitations and Future Directions

Even though this study was the first to consider adrenocortical interdependence and attunement across father-infant and mother-infant dyads during a laboratory stressor involving the SSP, there are a number of limitations to the current research that need to be noted. First, the results may not generalize to other studies using other infant-directed stress procedures such as the Still-Face Procedure (SFP) or arm restraints. The SSP involves physical separations between parent and infant and is often conducted during the latter half of the first year to assess the security of infant-parent attachments. Even though such separations between 12-month-old infants and their caregivers reliably elicit stress (Kuo et al., 2019; Spangler & Grossmann, 1993), infants of this age are becoming more independent as they enter toddlerhood, feeling more

comfortable exploring further away from their caregiver for longer periods of time, and developing more mature emotion and behavioral regulation strategies than younger infants. Young infants of around 4 to 6 months of age are more dependent on the caregiver to direct interaction and respond to affective and behavioral cues as has been so elegantly demonstrated in studies using the SFP. Infant cortisol reactivity has also been shown to decline with age (Egliston et al., 2007). Thus, our results may not be applicable to studies on parent-infant attunement among younger infants. We would not automatically expect similar findings relating to cortisol interdependence and attunement for younger infants and future research should be mindful of the rapid developmental changes occurring in the first year, rather than automatically assuming that cortisol interdependence and attunement, if evident, would be the same across different ages. Parents also do not appear to demonstrate a stress response to the SSP as infants do. This is not surprising considering that the SSP was developed to measure infant-attachment quality. The remaining question is then how do we attempt to measure cortisol interdependence and attunement between parents and their infants when infants are around 1 year of age and have developed an attachment relationship with their parents? Perhaps researchers should begin by studying attunement as a diurnal process between parents and infants to explore whether attunement exists prior to introducing an infant-directed stressor in an observational or experimental task that is not designed nor was ever intended to assess cortisol attunement.

Previous research has used procedures like the Still-Face Procedure (Provenzi et al., 2019; for review see Provenzi et al., 2016), Lab-TAB (e.g., Hibel et al., 2009), and a daily routine activity like a diaper change (Mörelius et al., 2012) to assess mother-infant cortisol attunement at various time points in infancy, but not father-infant cortisol attunement. Future research should consider the type of procedure used to elicit cortisol responses in parents and infants, and issues surrounding measurement equivalence for infants and parents, when considering the concept of attunement as it is difficult to argue for attunement between infants and parents when both are responding differently to the same means of measurement. The SSP was designed for use between 12 and 18 months (the age at which separation crying begins to decline; Jacobson & Wille, 1984) to assess the security of attachment, whereas the SFP has been used with 1- to 12-month-old infants to assess infant affective responses to an inattentive parent, neither of which were designed to assess cortisol attunement between parents and infants.

Second, infants in the current study were also from low-risk family environments, and the current findings may not reflect how parent-infant adrenocortical attunement may be altered for infants from impoverished, high-stress environments, or for infants at significant developmental risk. For instance, Provenzi and colleagues (2019) found that full-term compared to very preterm three-month-old infants displayed significant within-time cortisol correlations with their mothers in the SFP. Lastly, infants and their parents were from white, middle-class, two-parent, mother-father families in the U.S., and findings may not generalize to other family situations or to families from other cultural and ethnic backgrounds, although it should be noted that Abraham and colleagues (2014), using an Israeli sample, found that primary caregiver mother, secondary caregiver (heterosexual), and primary caregiver same-sex fathers demonstrated a similar "global parental caregiving brain network" contributing to parent-infant biobehavioral synchrony.

Conclusions

We found stronger support for *actor-only* models (i.e., infants' and parents' cortisol at one time point predicting subsequent cortisol) for both father-infant and mother-infant dyads during the SSP, suggesting there is greater stability in individual cortisol output over time, than the effect of one partners' cortisol on that of the other. We did find one significant partner path between infant T2 cortisol and parent T3 cortisol for both father-infant and mother-infant dyads; however, *post-hoc* exploratory models failed to uncover the mediating mechanisms linking infant and parent physiology for either father-infant or mother-infant dyads. Further research is clearly needed to determine whether the HPA axes of parents and infants are interrelated and if so, what accounts for these interdependent relations. Infant distress as exhibited during the SSP appeared to be a promising lead as it predicted infants' cortisol reactivity to separation, which, in turn, predicted the spillover of infant negativity displayed in subsequent mother-infant interactions, but we did not find similar findings for fathers and infants. We would conclude that the bio-behavioral relationship between infants and parents (both their fathers and mothers) is far more complex than what one might at first glance see as evidence of adrenocortical attunement between the HPA axes of infants and their adult caregivers based on simple within-time correlations between infant and parent cortisol. The underlying behavioral and affective processes linking cortisol in father-infant and mother-infant dyads may indeed be similar or different, but far more research using consistent definitions, methods, and statistical modeling is needed before we will have answers.

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Table 1

	1	2	3	4	5	6	7	8	9
1. Infant cortisol T1	.14	.70**	.74**	.34**	.29**	.42**	-.12	.02	.05
2. Infant cortisol T2	.92**	.12	.74**	.34**	.33**	.27*	-.00	.04	.07
3. Infant cortisol T3	.74**	.67**	.13	.42**	.25**	.26*	-.03	.07	.19
4. Parent cortisol T1	.26**	.28**	.31**	.04	.72**	.56**	-.10	-.03	-.04
5. Parent cortisol T2	.31**	.28**	.42**	.75**	.37*	.70**	.08	-.08	-.06
6. Parent cortisol T3	.33**	.29**	.39**	.62**	.80**	.39*	-.08	-.05	.01
7. Infant distress	.21	.33**	.17	.24*	.30**	.13	.39*	.13	.13
8. Infant negative mood	.06	.19	.04	-.05	-.06	-.07	.18	.19	-.32**
9. Parent sensitivity	-.16	-.15	-.24*	.10	.13	.01	.08	-.20	.32*
Mother-infant									
N	113	121	115	137	135	132	140	141	141
M	.19	.17	.16	.22	.15	.12	3.19	1.43	4.26
SD	.37	.27	.22	.14	.10	.09	1.23	.70	.78
Father-infant									
N	112	99	98	122	120	113	125	125	125
M	.17	.19	.13	.20	.14	.12	2.97	1.33	3.87
SD	.26	.28	.16	.14	.10	.08	1.29	.57	.91

Descriptives and Correlations of Study Variables for Parent-Infant Dyads Controlling for Time when Cortisol Collected

Notes. The values used here represent the raw data instead of the log transformed values. rs for the father-infant dyads are presented above the diagonal controlling for when cortisol was collected at T1, rs for the mother-infant dyads are presented below the diagonal controlling for when cortisol was collected at T1, and cross-dyad correlations are reported in the diagonal and underlined controlling for when cortisol was collected at T1 for both mother-infant SSP and father-infant SSP.
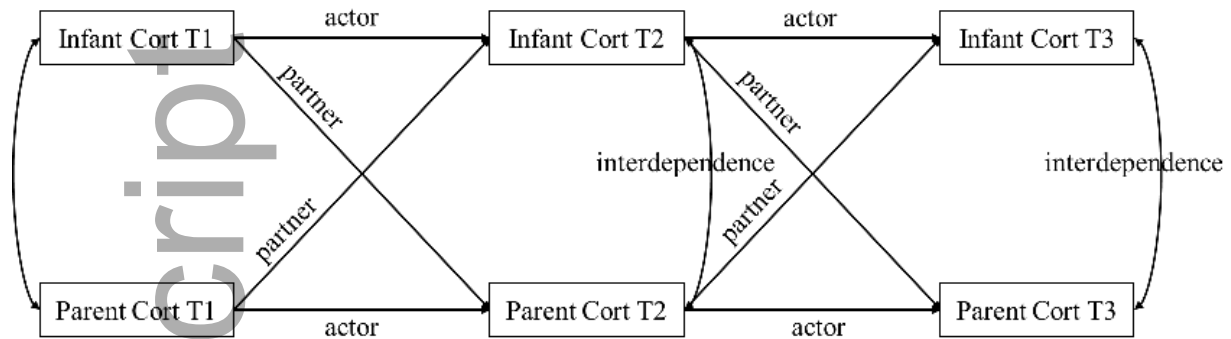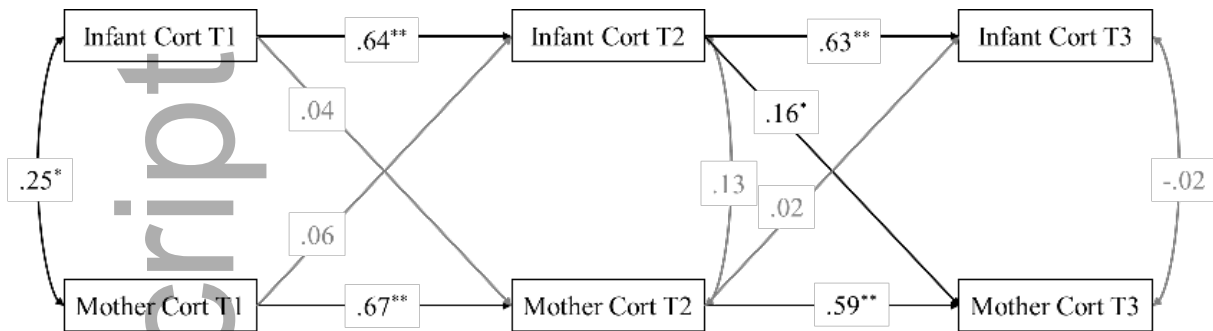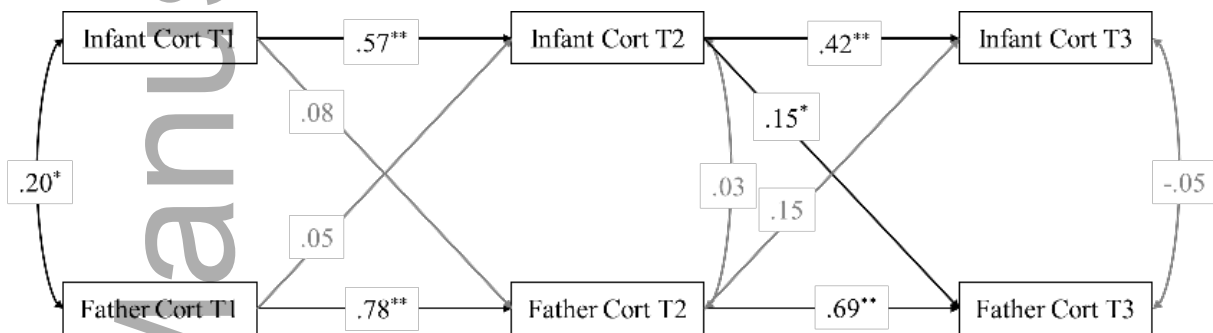
Figure 1

Conceptual Actor-Partner-Interdependence Model (APIM)

Figure 2

Infant and Mother Cortisol APIM

Infant and Father Cortisol APIM

Notes. Standardized coefficients are presented. Black lines represent significant paths, with grey lines representing non-significant paths.
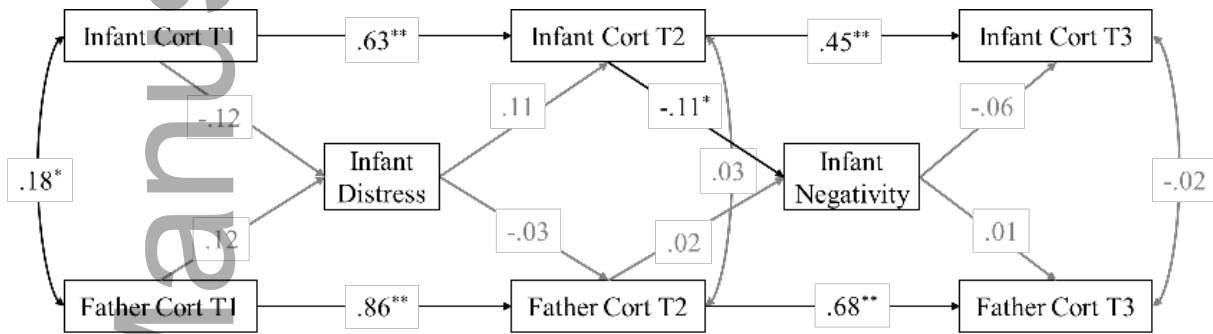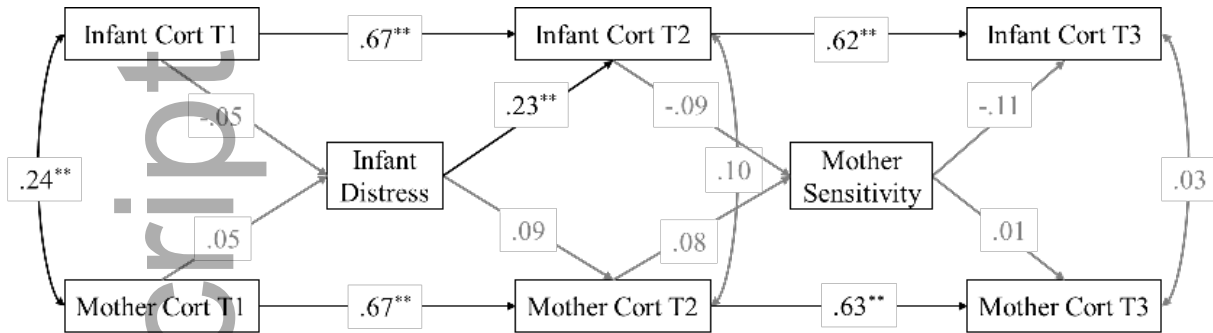*p < .05, **p < .01.

Figure 3

Mother-Infant Dyads: Infant Distress Model
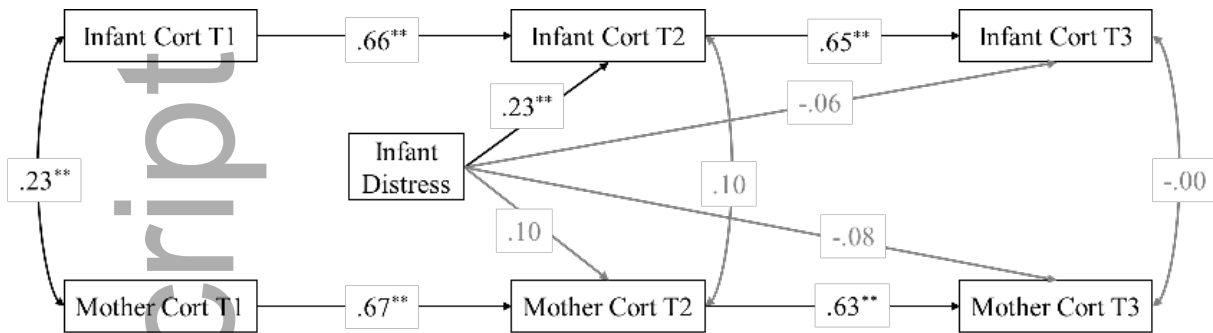
Father-Infant Dyads: Infant Distress Model
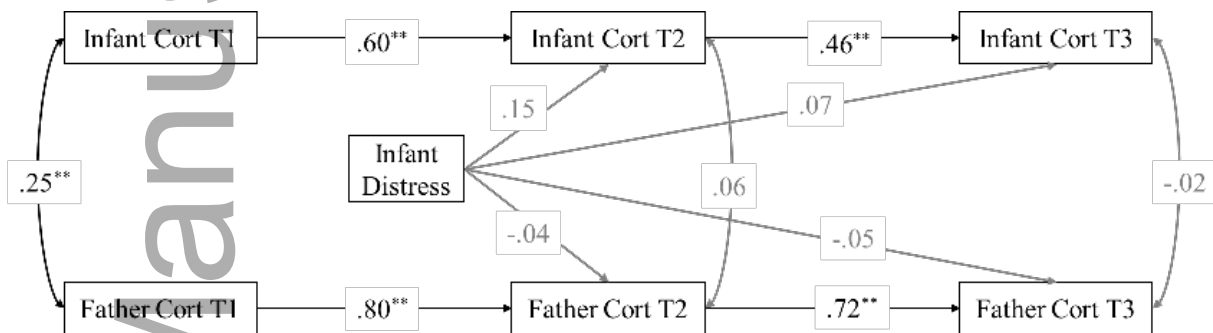
Notes. Standardized coefficients are presented. Black lines represent significant paths, with grey lines representing non-significant paths.
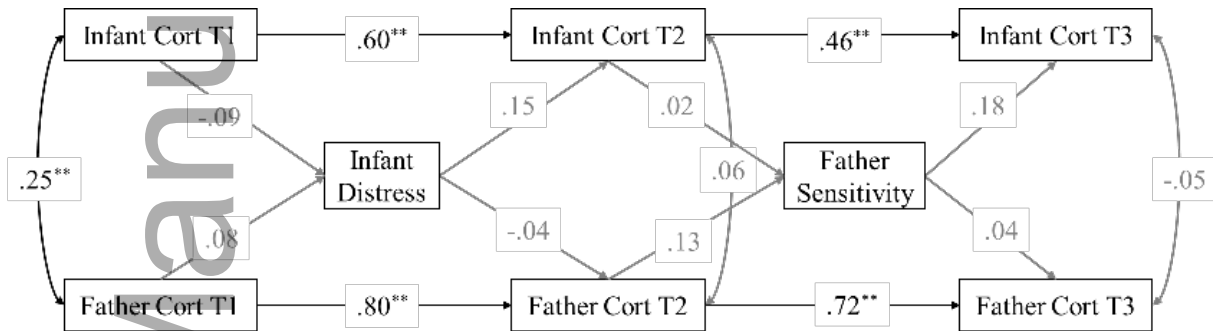* $p < .05$, ** $p < .01$.

Figure 4

Mother-Infant Dyads: Infant Affective Spillover Model
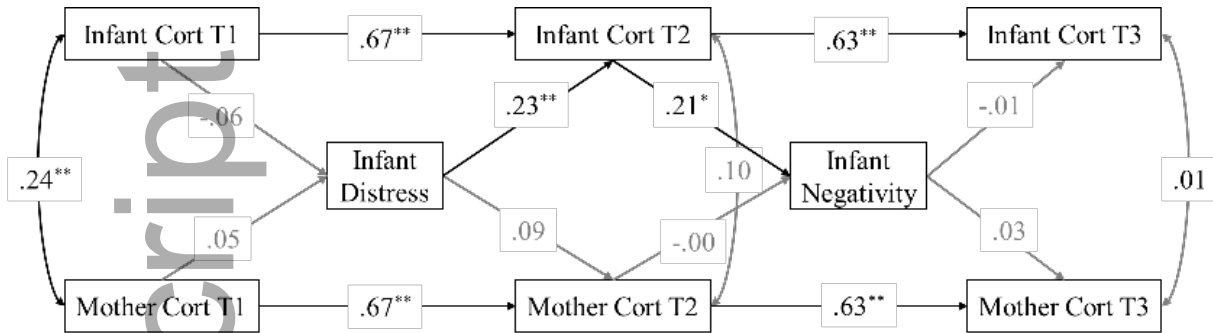
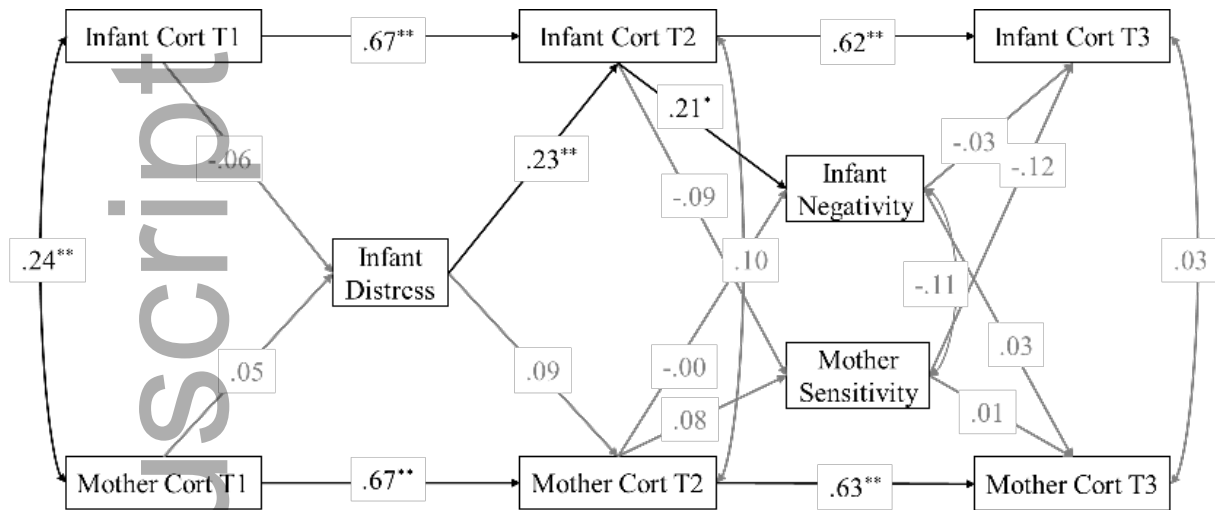
Father-Infant Dyads: Infant Affective Spillover Model

Notes. Standardized coefficients are presented. Black lines represent significant paths, with grey lines representing non-significant paths.
$^*p < .05, ^{**}p < .01$.

Figure 5

Mother-Infant Dyads: Parent Stress Model

Father-Infant Dyads: Parent Stress Model

Notes. Standardized coefficients are presented. Black lines represent significant paths, with grey lines representing non-significant paths.
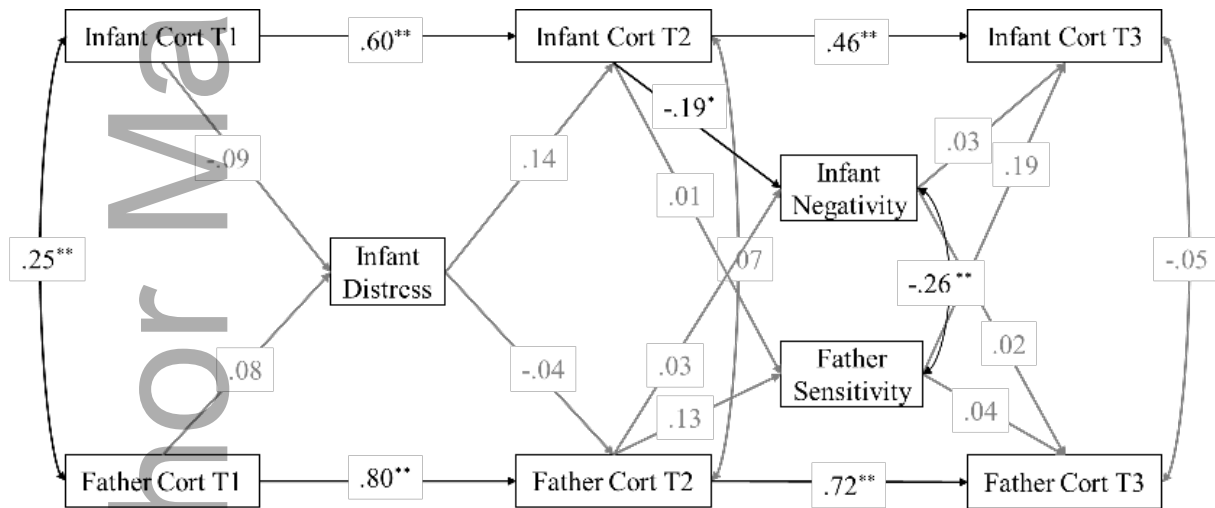$^*p < .05$, $^{**}p < .01$.

Figure 6

Mother-Infant Dyads: Parent-Infant Co-Regulation Model

Father-Infant Dyads: Parent-Infant Co-Regulation Model

Notes. Standardized coefficients are presented. Black lines represent significant paths, with grey lines representing non-significant paths.
*$p < .05$, **$p < .01$.