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Correlates and Consequences of Toddler Cortisol Reactivity to Fear

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Abstract

Cortisol reactivity to fear-eliciting stimuli in toddlerhood may represent an indicator of risk for anxiety-spectrum problems and other maladjustment. Thus, it is important to understand factors that may contribute to cortisol reactivity as well as those that determine its predictive relation to early emerging anxiety. In this vein, the current study investigated maternal comforting behaviors, both solicited and unsolicited by the toddler, as correlates of cortisol reactivity at age 2. Further, we investigated maternal comforting behaviors and behavioral indicators of fear in both a low-threat and a high-threat context as moderators of the relation between cortisol reactivity at age 2 and change in anxiety from age 2 to age 3. The sample comprised 99 2-year-old toddlers and their mothers. Toddlers provided saliva samples at baseline and after a fear-eliciting stimulus that were assayed for cortisol. Mothers were observed for comforting behavior while interacting with their toddlers in laboratory tasks and completed questionnaires about their toddlers' anxiety. Results indicated that unsolicited (spontaneous) comforting behavior related to toddler cortisol reactivity above and beyond solicited comforting and the level of fear toddlers displayed in the same task. Moreover, fear in a low-threat, but not high-threat, context moderated the relation between cortisol reactivity and change in anxiety, such that cortisol reactivity had a positive relation to anxiety at extreme levels of low-threat fear. Results suggest the importance of considering the caregiving environment and context-specific fear in understanding the nature of cortisol reactivity in the toddler years.

Cortisol reactivity to fear in early development has attracted scientific attention because of its predictive relation to anxiety problems and related risk factors (e.g., inhibited temperament). However, much is needed in terms of understanding developmental processes relevant to this biological response. For example, the caregiving environment may relate to variance in cortisol reactivity in early childhood, but a more nuanced understanding of specific parenting behaviors is warranted. Specifically, comforting behavior, although seemingly sensitive, has been linked to increased risk for anxiety when high levels are displayed in contexts that are objectively low in threat and may provide opportunities for toddlers to practice independent coping (Kiel & Buss, 2012). It remains unclear whether

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comforting behavior also relates to cortisol reactivity, and in what capacity. Theoretical models of the relation between control and risk for anxiety (Chorpita & Barlow, 1998) suggest that parenting behavior that is spontaneous rather than elicited by the child may be most related to a stress response, although empirical evidence for this distinction is needed. Further, the extent to which biological reactivity predicts anxiety development may depend on both maternal comforting behavior as well as other signs of maladaptive fear regulation. Fear expressions in low-threat contexts, rather than the higher threat contexts required to elicit a cortisol response, have been theorized and empirically shown to be a specific risk for anxiety in early childhood (Buss, 2011; Buss et al., 2013) and may be a necessary condition for cortisol reactivity to represent risk. Thus, to augment developmental models of cortisol reactivity to fear-eliciting situations and subsequent risk for anxiety, the current study examines maternal solicited and unsolicited comforting behaviors along with fear in a low-threat context in association with cortisol reactivity and as moderators of the predictive relation between cortisol reactivity and anxiety.

The Cortisol Response in Development

The physiological stress response is crucial in mobilizing the body's resources to respond to potentially threatening and challenging events. One component of this that has received a great deal of empirical attention is the cortisol response. Cortisol is a glucocorticoid and the end-product from a cascade of hormones secreted by the hypothalamic-pituitary-adrenal axis (HPA-axis; Gunnar & Quevedo, 2007). The peak cortisol response can be assessed from saliva samples 20 minutes after the presentation of a stressor. Although adaptive in moderation, the field has focused on the harmful physical and mental effects of extreme or prolonged activation of this system (McEwen & Stellar, 1993; McEwen, 1998). Due to inconsistent findings about what constitutes an adaptive cortisol response profile, debate continues about factors that may influence the development and long-term outcomes of this acute stress-response (Gunnar, 2001; Shirtcliff, Peres, Dismukes, Lee, & Phan, 2013). Theory from Boyce and Ellis (2005) proposes that increased biological activation and arousal can be either negative or positive depending on the child's environment. As such, contextual factors in a developing child's early environment and how they affect HPA-axis functioning should be investigated.

Numerous studies have attempted to identify the correlates and predictors of increased cortisol responses to stress. However, eliciting a cortisol response from laboratory stressors has proved challenging, particularly in toddlers (Gunnar, Talge, & Herrera, 2009). Traditionally-used stressor paradigms including a brief separation from a caregiver or exposure to a novel object or stranger have not reliably produced mean differences in cortisol (Gunnar et al., 2009). Such mixed findings may be related to the age of the child. Although infants under six months of age regularly demonstrate increased cortisol reactivity when faced with mild stressors like a medical exam or inoculation, this response begins to fade by the child's first birthday (Gunnar & Quevedo, 2007; Gunnar et al., 2009; Jansen, Beijers, Riksen-Walrave, & de Weerth, 2010). Various neurodevelopmental processes occur in these early years, and environmental factors like the quality of the caregiver-child relationship become more influential for the regulation of emotional arousal (Gunnar & Quevedo, 2007). Ursache, Blair, Granger, Stifter and Voegtline (2013) found that although

overall mean increases in cortisol were not observed with 2-year-old toddlers, their cortisol responses to fear specifically related to behavioral manifestations of fear. Thus, despite a non-significant mean-level difference across the sample, individual differences in reactivity were meaningful. This suggests that toddlers' fear displays could be used to verify that manipulations are effective in eliciting fear and thus, the cortisol response.

Even when taking this into consideration, to increase the likelihood of a meaningful cortisol response and related fear reaction, the fear-eliciting stimulus must be perceived as uncontrollable and unavoidable (Gunnar et al., 2009). Investigations have been conducted to determine the average amount of fear displayed by toddlers in response to standard, fear-eliciting stimuli (Buss, 2011). Findings indicate that the greatest number of toddlers display fear reactions in high-threat episodes, such as the episode from the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith & Rothbart, 1999) in which a large, remote-controlled spider approaches the toddler. This high intensity stressor is also relatively brief (< 30 seconds), so toddlers' distress reactions less frequently cause a premature removal of the stimulus. Such high-threat episodes seem most likely to produce both cortisol response and fear displays in two-year-olds (Gunnar, 2009; Ursache et al., 2013).

Certainly, outside of considering the best context for the display of cortisol reactivity, environmental influences may be influential in determining variance in this response. The parenting environment, in particular, has emerged as a salient influence on toddlers' cortisol reactivity to fear.

Parenting

The development of the physiological stress response can also be influenced by environmental experiences (Boyce & Ellis, 2005; Gunnar & Quevedo, 2007). Parenting behaviors are one salient mechanism through which children receive messages about how to interact appropriately with their surroundings. Parenting is particularly influential during early childhood when attachment relationships are forming and the neurological underpinnings of the stress response are canalizing. Specifically, early parenting experiences are shown to uniquely predict cortisol reactivity in a child nearly one year later (Blair et al., 2008). These findings demonstrate the lasting impact that parenting can have on the HPA-axis and warrant further research determining the parenting behaviors that are potentially beneficial or promote maladaptation in children.

Parenting that is harsh or overly intrusive communicates to a child that they lack mastery over their own environment and is thought to adversely affect the cortisol response (Gunnar & Quevedo, 2007). As parents employ strict discipline or overly controlling behaviors, children learn that they lack the ability to influence personal outcomes. Multiple studies have found that harsh or abusive parenting relates to increased cortisol reactivity in response to stressors (Dougherty, Klein, Rose, & Laptook, 2011; Gunnar & Quevedo, 2007; Hastings et al., 2011), as do high-conflict relationships (Rappolt-Schlichtmann et al., 2009). Further, toddlers of caregivers who were less supportive, more hostile, and intrusive demonstrated greater cortisol reactivity to a social challenge, particularly if the toddler was also inhibited

(Dougherty et al., 2011). Thus, the role of harsh parenting in the development of cortisol reactivity is well-established.

Comforting parenting behaviors may also undermine the development of coping behaviors. Although warmth and affection represent sensitive maternal responses in general, they also function to regulate young children's arousal, which may be adaptive when children need external support but maladaptive in situations in which the challenge is mild enough that children may have the opportunity to practice more independent coping. In a high-threat context, when the demands outweigh the child's coping resources, maternal comforting behavior is beneficial because it communicates support and warmth. However, in low-threat situations, comfort may communicate to children that they lack independent regulation skills and therefore impedes children's abilities to develop independent coping. Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss (1996) demonstrated that mothers of inhibited children who provided more comfort and encouraged approach had children who displayed more cortisol reactivity in the face of stress. Interestingly, cortisol levels post-stressor were unrelated to the child's comfort-seeking behaviors, so it appears that child cortisol reactivity was related to spontaneous, unsolicited maternal comforting behaviors. This is consistent with theory suggesting that a lack of control may be related to stress responses and anxiety (Chorpita & Barlow, 1998). Although solicited comforting (elicited by the child) has been related to anxiety development for more fearful children (Kiel & Buss, 2011; 2012), the uncontrollability of unsolicited comforting may be specifically linked to biological reactivity. To our knowledge, no research has determined if unsolicited comforting behaviors by parents predict a cortisol response independent of child distress displays. Thus, a primary goal of the current study was to determine whether unsolicited comforting during a low-threat situation related to cortisol reactivity above and beyond solicited comforting and toddlers' fear displays that could influence maternal responses.

Developmental models of cortisol reactivity to fear require understanding both predictors, such as the aforementioned aspects of parenting, as well as outcomes. Therefore, we now turn to conceptualizing the conditions under which cortisol reactivity to fear may predict risk for anxiety.

Understanding Implications of Cortisol Reactivity

Implications of cortisol reactivity may best be understood in the context of both the caregiving environment and broader characteristics of the child. Although a paucity of research exists demonstrating how the parenting context may impact the association between cortisol reactivity and future anxiety in children specifically, several studies have demonstrated how a host of parenting behaviors can moderate the relation between other behavioral risk factors and future anxiety development (Coplan, Arbeau, Armer, 2008; Davis & Buss, 2012; Degnan, Henderson, Fox, & Rubin, 2008; Mount, Crockenberg, Jò, & Wagar, 2010; Rubin, Burgess, & Hastings, 2002). The parenting behaviors investigated in these studies included unsolicited and inappropriately comforting, affectionate, and sensitive behaviors, all of which were found to strengthen the predictive association between various behavioral risk factors and future anxious and withdrawal symptoms across early and middle childhood. Thus, it is clear that the parenting context can influence how risk factors affect

anxiety outcomes in young children, but it remains to be determined how cortisol, as a risk factor, is impacted by inappropriately comforting parenting, specifically, to predict internalizing symptoms in preschoolers. However, investigating this moderation without considering how child characteristics may impact this relation may not yield a comprehensive picture the most salient influences.

Temperament theory suggests that children are born with dispositional tendencies to react to the environment (Goldsmith et al., 1987). Of particular interest within the scope of risk for anxiety are toddlers who display an inhibited or fearful temperament. This temperamental style reflects an underlying biological basis for patterns of behavior and reactivity when exposed to novelty and unpredictability (Kagan, Snidman, Arcus, & Reznick, 1994). Children who are inhibited may have a lower threshold for arousal and respond with greater distress and avoidance in unfamiliar situations (Kagan et al., 1994). Temperamental inhibition relates to increased risk for a variety of anxiety symptoms and internalizing problems (Biederman et al., 2001).

Given moderate estimates of stability of inhibited temperament across development (Rubin, Hastings, Stewart, Hendersen, & Chen, 1997; Rubin, Burgess, & Hastings, 2002), there has been increased interest in determining how to identify fear expressions that most specifically identify risk for anxiety. Some have speculated that only those extremely inhibited children (top 15–20%) will be consistently inhibited across situations and time (Kagan, 1989). More recently, Buss and colleagues (Buss, 2011; Buss et al., 2013) demonstrated that the eliciting context of fear must be considered, as children who are extremely fearful and avoidant in objectively less threatening contexts are more likely to display social anxiety symptoms in kindergarten. Further, fear in lower-threat contexts more specifically identified risk than high levels of fear in higher-threat contexts that are typically used to measure inhibited temperament. Buss (2011) categorized contexts as low-threat by determining the average amount of fear displayed by toddlers in relation to other novelty episodes. This work found that high fear in low-threat contexts was essential in characterizing a pattern of dysregulated fear that is maladaptive, as compared to perhaps a more adaptive response of expressing fear in more highly threatening situations.

Cortisol reactivity may be particularly predictive of anxiety-spectrum outcomes when observed in conjunction with dysregulated fear. Evidence exists suggesting that inhibited toddlers, as traditionally assessed, may display greater cortisol reactivity in novel situations (Gunnar, Tout, de Haan, Pierce, & Stansbury, 1997; Kagan, Reznick, & Snidman, 1987; Talge, Donzella, & Gunnar, 2008), although this has not been consistently shown (Donzella, Gunnar, Krueger, & Alwin, 2000). Perhaps these mixed findings for inhibition reflect an inadequate consideration of the context in which the distress was displayed. Indeed, in one study, cortisol reactivity was shown to be highest in relation to fear expressed in a lower-threat versus higher-threat stranger approach (Buss, Davidson, Kalin, & Goldsmith, 2004). Although fear in low-threat conditions and cortisol reactivity relate to one another and independently relate to anxiety, it remains unclear how they may work in conjunction to predict anxiety. Given that cortisol reactivity is inconsistently related to risk, it seems likely that it would be most predictive when it is considered in the context of other indicators of a broader profile of risk.

Thus, the extant literature suggests, but has not tested whether either comforting behavior or behavioral indicators of temperament, especially dysregulated fear, influence whether heightened cortisol reactivity predicts increased anxiety over time. As such, the second major aim of this study was to determine if either solicited or unsolicited comforting behavior, or extreme fear in a low-threat context is a necessary condition for cortisol reactivity to predict the development of early anxiety problems.

The Current Study

The current study aimed to clarify both correlates and consequences of toddler cortisol reactivity to fear. Given prior work showing that meaningful cortisol responses may best be elicited in toddlerhood by higher-threat stimuli, we examined cortisol reactivity in response to the remote-controlled spider laboratory task. However, comforting behaviors may be most meaningful when displayed in a low-threat laboratory context, which putatively reflect mothers' tendencies towards external management of toddlers' emotion regulation in situations in which the toddler may benefit from practice in independent coping. According to theory that uncontrollable aspects of the environment may be particularly relevant to stress reactivity (Chorpita & Barlow, 1998), we aimed to examine the unique relation between unsolicited comforting behavior and cortisol reactivity. Toddlers' fear reactions in this low-threat context may both influence how mothers behave (both in reaction to toddlers' bids for support as well as spontaneously) and relate to cortisol reactivity themselves. Thus, we hypothesized that unsolicited comforting behavior in a low-threat task would uniquely relate to increased cortisol reactivity above and beyond solicited comforting behavior and toddler fear in the same situation.

Second, we hypothesized that the predictive association between cortisol reactivity and anxiety would be moderated by fear displayed in a low threat context, such that higher levels of cortisol reactivity would predict higher anxiety when toddlers also displayed high levels of fear, and by solicited and unsolicited maternal comforting behavior, such that cortisol reactivity would predict anxiety at high levels of each type of comforting. To further validate the unique role of fear in low-threat contexts, we examined fear in a high-threat context as a moderator of the relation between cortisol reactivity and anxiety. Although we did not expect this moderation to occur, we refrain from offering a hypothesis of the null.

Method

Participants

The larger sample from which these data were derived comprised 117 2-year-old toddlers and their mothers. The current study focuses on the 99 toddlers (41 female; M_{age} = 24.78 mo., SD_{age} = 0.73 mo.) who contributed at least one saliva sample that was able to be assayed. Families were recruited through direct mailings informed by locally published birth announcements (n = 86) and in-person at the Women, Infants, and Children's (WIC) program (n = 13). WIC is a government-subsidized program that provides education and assistance to low-income mothers who are pregnant and/or have children under 5 years of age. Toddlers were primarily (81%) European American, with Asian American (8%), African American (7%), biracial (2%), and "other" (2%) racial backgrounds represented; 6%

were reported to be Hispanic/Latino. Socioeconomic status (SES) was assessed using the Hollingshead four-factor index, which quantifies mothers' and fathers' (if available) education levels and occupations, resulting in a scale ranging from 8 to 66. The current sample was on average middle class (Hollingshead *Mean* = 49.33, *SD* = 12.26), with a range of SES represented (*Range* = 17 to 66). Sixty-nine participants participated in a follow-up when children were approximately 3 years of age.

Procedures

Mother-toddler dyads participated in a laboratory visit following a within-subjects design. Prior to arriving at the laboratory, mothers provided written informed consent and completed a battery of questionnaires including a measure assessing her toddler's anxiety. Upon arriving, a primary experimenter fielded any questions about consent and explained all procedures to the mother. Toys were available for the toddler to play with while the mother and experimenter talked. After the toddler acclimated to the laboratory (at least 20 minutes after arrival), a baseline saliva sample was taken. The toddler was asked to mouth a synthetic swab until it was saturated with saliva.

As part of a larger battery of tasks (see *citation blinded*), dyads participated in *Clown* and *Spider* episodes. For both episodes, mothers were instructed to behave "however you typically would or seems natural to you." In Clown, the primary experimenter showed the toddler and mother into a room and then exited. Shortly after, a secondary experimenter dressed in a clown costume, colorful wig, and red nose entered the room. With a friendly demeanor, she invited the toddler to play three games (blowing bubbles, catch with beach balls, musical instruments) that lasted 1 minute each. She removed her wig and nose after the second game. After the games, she invited the toddler to help pick up her toys and then exited the room. In Spider, the mother was asked to bring her toddler into the room and sit the toddler on her lap in a chair in one corner of the room. In the opposite corner was a large plush spider affixed to a box covering a remote-controlled truck. After the primary experimenter left the room, the spider was controlled by remote from behind a one-way mirror in the following pattern: approached half-way towards the dyad and paused for 10 seconds, retreated to its starting place and paused 10 seconds, approached all the way and paused 10 seconds, then retreated to its original place. The primary experimenter then returned and asked the child to come touch the spider with up to three prompts. Whether or not the child touched it, the experimenter touched the spider and stated it was a pretend spider. Several episodes that were more neutral in nature occurred in between the Clown and Spider episodes. Because of our interest in individual differences, episodes proceeded in the same order across participants. Notably, in an independent sample, counterbalancing these tasks did not result in any differences in toddler fear across conditions (*citation blinded*).

Episodes were recorded for later scoring of toddler fear and maternal comforting behaviors. Coders were trained in scoring systems for each behavior that have previously been used by the first author in this as well as independent samples (*citations blinded*). Coders were required to achieve adequate reliability (intraclass correlation coefficient [ICC] > .80) with a master coder (first author) prior to coding independently. Additional reliability checks occurred throughout coding to prevent coder drift. Final reliability estimates are reported in

descriptions of the measures. Different coders scored toddler fear and maternal comforting behaviors to minimize bias.

Toddlers provided a post-visit saliva sample approximately 20 minutes after completion of the Spider episode. Mothers were also sent home with supplies to collect an additional saliva sample from their toddlers approximately 20 minutes after they left the visit. They were asked to return this sample to the lab using a provided preaddressed, prestamped envelope. Cortisol concentrations from this home sample were used in the imputation of missing baseline or post-visit cortisol values. Synthetic swabs were stored in plastic tubes and stored at −50° C until shipped on dry ice to the University of Trier for analysis.

When toddlers were approximately 3-years-old, mothers were asked about their interest in completing a follow-up battery of questionnaires that included the same measure of toddler anxiety. Interested mothers completed a new written consent form and the questionnaires through the mail.

Measures

Toddler fear—Given that fear is reflected in both affective expressions and behavior, toddlers were scored for both negative affect (NA) and shyness/withdrawal, each on a 1 (*no display*) to 5 (*extreme or prolonged display*) scale in the Clown and Spider episodes. NA was coded for displays of crying/fussing as well as all facial expressions of negative valence, as toddlers use affective displays beyond fear to communicate distress in these contexts (Buss & Kiel, 2004). Shyness/withdrawal included displays of wariness, retreat, reductions in activity, and avoidance of the stimulus. Coders provided one score for NA and one score for shyness for each episode in order to holistically describe the intensity of the toddler's expression and behavior in that episode. NA (ICC = .84) and shyness (ICC = .78) scores were substantially related within both Clown (r = .55, p < .001) and Spider (r = .64, p < .001), so they were aggregated into a fear variable by computing their mean for each episode, hereafter referred to as *low-threat fear* and *high-threat fear*.

Maternal comforting behavior—Mothers were scored for the extent to which they physically or verbally comforted or soothed their toddlers on a 0 to 3 scale (0 = *none*, 1 = *touches child or says something comforting*, 2 = *actively soothes child [rubs arm or back]*, 3 = *hugs or embraces child*) each 10 second epoch of the Clown episode (ICC = .95). For each nonzero score, coders also noted whether the comforting was in response to the child's bid for comfort ("solicited") or was spontaneous ("unsolicited") (100% inter-rater agreement). Epoch scores were averaged across the episode separately for *solicited comforting* and *unsolicited comforting*.

Cortisol reactivity—Prior to completing any analyses, cortisol distributions were assessed for skew and kurtosis. Due to positive skew (all values >1.0), cortisol levels were log10 transformed (Gunnar, Morrison, Chisholm, & Schuder, 2001). Of the 99 toddlers in the sample, 77 toddlers (71.71%) provided usable saliva samples at both baseline and post-visit. Multiple (10) imputation techniques were used to replace missing data. Cortisol concentrations appeared to be different for toddlers using allergy medicine (n=1) and pain medicine (n=1). No significant differences emerged for toddlers based on time since waking,

time of day of the visit, the presence of any illness, infection, or due to any other medication use. The use of allergy or pain (e.g., ibuprofen) medication was dummy coded and included in the imputation algorithm. Contemporary approaches to handling missing data suggest it is useful to include auxiliary variables that are not included in primary analyses yet relate to variables of interest in the imputation algorithm (Graham, 2009). Therefore, cortisol levels from the home saliva sample were also included.

Post-visit saliva samples were then regressed on baseline saliva samples and unstandardized residuals were calculated. The uses of allergy and pain medications were included as independent variables in the regression equation. Positive residuals indicate greater cortisol reactivity and negative residuals indicate less cortisol reactivity. This measure provides a continuous measure of HPA-axis reactivity.

Toddler anxiety—Mothers completed the Infant-Toddler Social Emotional Adjustment Scale (ITSEA; Carter & Briggs-Gowan, 2000), a 193-item measure assessing both typical and atypical behaviors seen in 18- to 36-month-olds. Mothers are asked to respond how true various descriptions of behaviors are for their children on a 3-point scale (0 = *not true or rarely true*; 1 = *somewhat or sometimes true*; 2 = *very true or often true*). The ITSEA has demonstrated adequate internal consistency, test-retest reliability, and construct and criterion validity (Carter, Briggs-Gowan, Jones, & Little, 2003). The current study formed a composite of items from the General Anxiety (10 items; e.g., “Seems nervous, tense, or fearful”) and Separation Distress (6 items; e.g., “Cries or hangs onto you when you try to leave”) scales. Mothers completed the scale both at both the age 2 (alpha = .64) and age 3 (alpha = .64) assessments.

Results

Missing Data

Of the 99 participants, 5 (5%) were missing the age 2 ITSEA and 30 (30%) were missing the age 3 ITSEA. Little’s MCAR test suggested that the pattern of missing data did not significantly deviate from the Missing Completely at Random pattern ($\chi^2[15] = 23.97$, $p > .05$). However, at both time points, those participants missing the ITSEA had reported lower SES (Age 2: $M = 35.80$, $SD = 14.18$; Age 3: $M = 42.77$, $SD = 14.91$) than those who were not missing it (Age 2: $M = 50.05$, $SD = 11.80$; Age 3: $M = 52.18$, $SD = 9.72$; ts = 2.61, 3.74, respectively, ps < .05; Cohen’s $d = 1.09, 0.75$, respectively). They did not differ on any other measures. Consistent with contemporary recommendations for handling missing data (Graham, 2009), SES was included in the algorithm (along with cortisol reactivity, low-threat and high-threat fear, maternal solicited and unsolicited comforting behaviors, existing values of the ITSEA anxiety composite at age 2 and age 3, and the cortisol reactivity by low-threat fear interaction) when we imputed missing values across 20 imputations as well as primary analyses using the ITSEA as the dependent variable. Consistent with other developmental studies (e.g., Dollar & Stifter, 2012), we averaged values across the 20 imputations to derive our final measures of age 2 anxiety and age 3 anxiety.

Preliminary Results

Descriptive statistics and bivariate relations are presented in Table 1. The dependent variables for primary analyses (cortisol reactivity and age 3 anxiety) demonstrated reasonable adherence to normal distributions (skew < |2.00|). The correlation between high-threat fear and cortisol reactivity served as a manipulation check and indicated that toddlers exhibiting higher fear in this episode also evinced higher cortisol reactivity. Female toddlers expressed more high-threat fear (M = 3.23, SD = 1.04) than boys (M = 2.75, SD = 1.23; t[97] = 2.15, p < .05, Cohen's d = 0.42). Gender differences did not exist for the primary outcome measures of cortisol reactivity or anxiety (ts < 1.90, ps > .05), and gender was not of primary interest in the current study; thus, it was not considered further.

Maternal Comforting Behavior and Toddler Cortisol Reactivity

Evident in bivariate correlations was the relation between unsolicited comforting and cortisol reactivity (Table 1). Solicited comforting had no relation to cortisol reactivity. Toddler low-threat fear related to both solicited and unsolicited maternal comforting behavior. Given this shared variance, correlations cannot determine whether unsolicited comforting behavior, unique from the toddler fear to which it may be a reaction, relates to cortisol reactivity.

In order to examine whether maternal unsolicited comforting behavior related uniquely to cortisol reactivity, we ran a multiple regression analysis including maternal unsolicited comforting, maternal solicited comforting, and toddler low-threat fear as predictors and cortisol reactivity as the dependent variable. As can be seen in Table 2, unsolicited comforting behavior emerged as significant in relation to cortisol reactivity. This suggests that, even when considering toddlers' fear and the comfort they directly elicit, spontaneous comforting behavior in less threatening contexts relates to a heightened cortisol response to a more stressful situation.

Longitudinal Prediction of Anxiety from Toddler Cortisol Reactivity and Fear

To address our second hypothesis, that toddler cortisol reactivity would predict anxiety development most strongly in the context of an indicator of dysregulated fear (i.e., high fear in a low-threat context) and high levels of maternal comforting behavior, we conducted moderation analyses. We ran two models, one including low-threat fear and the second including high-threat fear. Fear and maternal comforting variables were centered at their means, and cortisol reactivity was already centered at its mean as a residual variable. Interaction terms were computed as the cross-product of the relevant moderator and cortisol reactivity. A multiple regression model with age 3 anxiety as the dependent variable included the predictor of cortisol reactivity; the moderators of the specified fear variable, maternal solicited comforting, and maternal unsolicited comforting; and the three interaction terms as predictors. Age 2 anxiety was included so that other predictors could be interpreted as predicting change in anxiety. SES was included as a covariate. All predictors, including interaction terms, were entered simultaneously into the relevant regression model. A significant interaction was probed by recentering the moderator at various points along its distribution as well as determining the region of significance, or the value of the moderator

at which cortisol reactivity shifted from having a non-significant to significant relation to age 3 anxiety.

In the low-threat fear model, the cortisol reactivity X low-threat fear interaction was significant in relation to anxiety (Table 3). Probing revealed that cortisol reactivity did not predict anxiety at low (−1 SD; $b = -0.23$, $SE = 0.14$, $\beta = -0.27$, $t = -1.65$, $p = .102$, 95% CI [−0.51, .05], $sr^2 = .021$) or mean low-threat fear (Table 3). It had a marginally significant relation at a high value (+1 SD) of low-threat fear ($b = 0.21$, $SE = 0.11$, $\beta = 0.25$, $t = 1.98$, $p = .051$, 95% CI [−0.00, 0.42], $sr^2 = .030$) and a significant positive relation at a very high (+2 SD) value of low-threat fear ($b = 0.43$, $SE = 0.18$, $\beta = 0.51$, $t = 2.42$, $p = .018$, 95% CI [0.08, 0.79], $sr^2 = .045$). The region of significance suggested that the simple slope of cortisol reactivity shifted to significance at a value of low-threat distress of 3.01, or 1.01 SD above the mean. Therefore, when displayed by a toddler who exhibits a high level of fear in a relatively unthreatening context, cortisol reactivity is a meaningful predictor of increased anxiety across toddlerhood.

To test whether this interactive effect was specific to low-threat fear, we repeated this analysis, this time using high-threat fear. Given that neither of the comforting behavior moderators reached significance in the previous model, they were left out of this one. In this model (Table 3), the interaction was not significant. Thus, only high fear in the low-threat condition provided the necessary condition under which cortisol reactivity associated with high-threat fear predicted anxiety development.

Discussion

The current study aimed to identify specific parenting correlates as well as determinants of the consequences of toddler cortisol reactivity to fear. Meaningful individual differences in cortisol reactivity were elicited by a standardized laboratory task previously determined to be “high-threat” (Buss, 2011; Goldsmith & Rothbart, 1999), further validated in the current study by an association with toddlers’ fear expressions in that task. We found that unsolicited comforting in a standardized “low-threat” context (Buss, 2011) uniquely related to toddler cortisol reactivity, above and beyond comforting directly solicited by the toddler and toddler fear in that context. Finally, toddlers’ low-threat fear moderated the association between cortisol reactivity and anxiety, such that, only for toddlers exhibiting extreme fear in the low-threat context, cortisol reactivity predicted anxiety one year later, controlling for current anxiety. Fear in the high-threat context did not impact anxiety development. We believe these results offer new insights into the development and outcomes of toddler cortisol reactivity to fear.

Previous theories of stress and anxiety suggest that uncontrollability is a key feature of environmental influence on these outcomes (Chorpita & Barlow, 1998). Consistent with this, it was spontaneous maternal comforting, rather than comforting that toddlers elicited, that related to cortisol reactivity to a more stressful and fear-eliciting context. The next step in this line of research would be to understand the mechanism explaining this association. This seems particularly important given that these results, in one regard, may be counterintuitive to generally accepted knowledge that comforting behavior is indicative of

sensitive parenting, which should be adaptive for children's stress reactivity and broader social-emotional outcomes. However, in the context of fear, specifically, there is increasing evidence that parenting behavior that appears "sensitive" in most contexts does not function the same way. For example, curvilinear effects have been found, such that both low and very high levels of sensitivity relate to anxiety (Mount et al., 2010). When parents provide external management of children's stress regulation, children do not have to engage in active coping. This may be beneficial in contexts that present a level of challenge that surpasses the child's current abilities, but in more mildly challenging situations like our low-threat context, children may have to work to regulate independently, but it is within the range of their regulatory abilities and so serves as important practice and building of mastery over the environment. This is consistent with work by Rubin, Cheah, and Fox (2001) showing that maternal oversolicitous (overly warm, controlling) behavior in a less, but not more, challenging task related to children's social reticence. Perhaps this overly involved behavior represents a pattern established in the caregiver-child relationship that communicates that the child is not capable of handling minor stressors. An undermined sense of control may then lead to cognitive vulnerabilities related to anxiety (Chorpita & Barlow, 1998) that represent difficulties in regulating negative emotions (Thompson, 2001). As such, children become more nervous when presented with novel and potentially distressing situations. This adds to findings by Nachimas and colleagues (1996) that maternal comforting related to increased reactivity but only with inhibited children, whereas our current findings were across a general sample. Thus, toddlers' emerging emotion regulation abilities may be the mechanism of this relation. Future research will need to empirically test this hypothesis.

It would also be informative to understand maternal characteristics that predict this pattern of parenting behavior as well as potential moderators of this association to more precisely identify dyads at risk for maladaptive processes. Maternal cognitions (e.g., parenting goals, attitudes about and attunement to fear) and physiology have previously been suggested as determinants of similar parenting behavior (Coplan, Lagacé-Séguin, & Moulton, 2002; Kiel & Buss, 2012). Investigating whether these specifically relate to unsolicited comforting behavior will be a task for future research.

It is also noteworthy that spontaneous comforting related to toddler cortisol reactivity given that solicited comforting has also been shown to play a role in children's anxiety development. In this case, more temperamentally inhibited toddlers may be eliciting comfort and protection from their mothers, and this reinforces these children's dependence on external management of their stress and emotions in a cyclical fashion (Dadds & Roth, 2001; Kiel & Buss, 2011; 2012). Rather than anxiety more broadly, the current study focused on cortisol reactivity, for which different aspects of parenting may be more salient. Further investigation into the consequences of both spontaneous and elicited comforting behavior in contexts suitable to independent regulation is warranted. Moreover, these results have clinical implications, such that it may be beneficial for early intervention to focus on parenting behaviors that relate to toddlers' physiological arousal.

The fact that only fear in the low-threat, and not the high-threat, context moderated the association between cortisol reactivity and increased anxiety is consistent with recent theory and empirical findings on the construct of dysregulated fear (Buss, 2011; Buss et al., 2013).

This work suggests that children who display fear in higher threat contexts will be a heterogeneous group because these contexts more universally elicit a fear response that is putatively adaptive. Only low-threat contexts highlight dysregulation because the fear does not match the properties of the eliciting context. In the Clown episode, the stimulus was very friendly and engaged in activities that are familiar to most toddlers. Unlike the spider, she also remained on the other side of the room, providing toddlers with control over the proximity within which they interacted with her. Certainly, besides the intensity of threat of the episodes, the tasks differed in other ways. The Clown episode was more social than the Spider episode, although the latter did have a social component when the experimenter returned and asked the toddler to engage with the spider. It would be beneficial for future work to further disentangle these elements to more precisely identify the features of the contexts that highlight risk for anxiety.

Importantly, cortisol did not predict anxiety development on its own. Rather, only in the context of low-threat fear was it a significant predictor of anxiety. This may also relate to issues of context. Although cortisol reactivity has been associated with temperamental fear and inhibition that is linked to risk for anxiety, cortisol reactivity can also represent an adaptive response to threat. Thus, in a higher-threat context, toddlers exhibiting high cortisol reactivity may again be a heterogeneous group comprising children with both sensitivity and more typical thresholds of activation of the amygdala and HPA axis. When cortisol reactivity occurs in the context of behavioral indicators of a lower threshold for arousal (i.e., high fear in low threat), it may be an indicator of a coherent profile of fear dysregulation. Future work using person-centered approaches to develop psychobiological profiles of anxiety risk that use multiple levels of analysis including both biology and behavior, as well as environment and culture, would help to further specify the role cortisol reactivity may play.

The relation between cortisol reactivity and future anxiety was not moderated by maternal comforting behavior. It is possible that parenting plays a stronger moderating role when predicting toddlers' anxiety from previous behavior rather than biology, although the interaction between biology and the caregiving environment in relation to developmental outcomes remains a burgeoning area of research (Calkins, Propper, & Mills-Koonce, 2013). Potentially, other caregiving behaviors beyond comforting are more relevant moderators.

Limitations of the current study should be considered when interpreting our results and point to additional areas for future research. The sample was fairly homogenous, representing a primarily middle class and European American population. Both parenting and fearful behavior have different implications in different cultures, both within North America and between the United States and other countries (Belsky & Jaffee, 2006; Chen et al., 1998; Hill & Bush, 2001). Further, we focused solely on mothers. Fathers play important roles in children's development, both along typical trajectories and in terms of anxiety development (Edwards, Rapee, & Kennedy, 2010). Whether our results extend to both mothers and fathers of diverse backgrounds should be directly tested to understand the boundaries of the generalizability of our findings. Also, we assessed parenting behavior in one context that putatively represents broader patterns that impact children's development. It would be beneficial for research continuing along these lines to examine parenting in a variety of

contexts both inside and outside of the laboratory to more comprehensively understand parent-child dynamics related to cortisol reactivity. It would also be useful to directly test cortisol reactivity to the Clown episode, although our current approach is in line with recommendations for assessing cortisol reactivity in toddlers. Finally, we relied on a maternal report measure of anxiety. Although controlling for age 2 anxiety represented a methodological strength, and shared method variance was not a concern given that no other variables were derived from maternal report, it will be important to replicate these results using other methodologies of anxiety assessment.

In sum, the current study extended previous work on toddlers' psychobiological reactions to fear. Our results suggest that both parenting and the context of fear are important to consider in developing a better understanding of correlates, potential contributions, and outcomes of cortisol reactivity during early development.

References

Belsky, J.; Jaffee, SR. The multiple determinants of parenting. In: Cicchetti, D.; Cohen, DJ., editors. Developmental psychopathology, Vol. 3: Risk, disorder, and adaptation. 2. Hoboken, NJ: John Wiley & Sons, Inc; 2006. p. 38-85.

Biederman J, Hirshfeld-Becker DR, Rosenbaum JF, Hérot C, Friedman D, Snidman N, Faraone SV. Further evidence of association between behavioral inhibition and social anxiety in children. American Journal of Psychiatry. 2001; 158(10):1673–1679. [PubMed: 11579001]

Blair C, Granger DA, Kivlighan KT, Mills-Koonce R, Willoughby M, Greenberg MT, Fortunato CK. Maternal and child contributions to cortisol response to emotional arousal in young children from low-income, rural communities. Developmental Psychology. 2008; 44(4):1095–1109. [PubMed: 18605837]

Boyce WT, Ellis BJ. Biological sensitivity to context: I. An evolutionary–developmental theory of the origins and functions of stress reactivity. Development and Psychopathology. 2005; 17(02):271–301. [PubMed: 16761546]

Buss KA. Which fearful toddlers should we worry about? Context, fear regulation, and anxiety risk. Developmental Psychology. 2011; 47(3):804–819. [PubMed: 21463035]

Buss KA, Davidson RJ, Kalin NH, Goldsmith HH. Context-specific freezing and associated physiological reactivity as a dysregulated fear response. Developmental Psychology. 2004; 40(4): 583–594. [PubMed: 15238045]

Buss KA, Davis EL, Kiel EJ, Brooker RJ, Beekman C, Early MC. Dysregulated fear predicts social wariness and social anxiety symptoms during kindergarten. Journal of Clinical Child & Adolescent Psychology. 2013; 42(5):603–616. [PubMed: 23458273]

Buss KA, Kiel EJ. Comparison of sadness, anger, and fear facial expressions when toddlers look at their mothers. Child Development. 2004; 75:1761–1773. [PubMed: 15566378]

Calkins SD, Propper C, Mills-Koonce WR. A biopsychosocial perspective on parenting and developmental psychopathology. Development and Psychopathology. 2013; 25:1399–1414. [PubMed: 24342847]

Carter, AS.; Briggs-Gowan, M. Unpublished manual. University of Massachusetts Boston Department of Psychology; Boston, MA: Yale University; New Haven, CT: 2000. The Infant-Toddler Social and Emotional Assessment (ITSEA).

Carter AS, Briggs-Gowan MJ, Jones SM, Little TD. The Infant-Toddler Social and Emotional Assessment (ITSEA): Factor structure, reliability, and validity. Journal of Abnormal Child Psychology. 2003; 31:495–514. [PubMed: 14561058]

Chen X, Hastings PD, Rubin KH, Chen H, Cen G, Stewart SL. Child-rearing attitudes and behavioral inhibition in Chinese and Canadian toddlers: A cross-cultural study. Developmental Psychology. 1998; 34:677–686. [PubMed: 9681259]

Chorpita BF, Barlow DH. The development of anxiety: The role of control in the early environment. Psychological Bulletin. 1998; 124(1):3–21. [PubMed: 9670819]

Coplan RJ, Arbeau KA, Armer M. Don't fret, be supportive! Maternal characteristics linking child shyness to psychosocial and school adjustment in kindergarten. Journal of Abnormal Child Psychology. 2008; 36(3):359–371. [PubMed: 17899358]

Coplan RJ, Hastings PD, Lagacé-Séguin DG, Moulton CE. Authoritative and authoritarian mothers' parenting goals, attributions, and emotions across different childrearing contexts. Parenting: Science and Practice. 2002; 2:1–26.

Dadds, MR.; Roth, JH. Family processes in the development of anxiety problems. In: Vasey, MW.; Dadds, MR., editors. Developmental psychopathology of anxiety. New York: Oxford University Press; 2001. p. 278-303.

Davis EL, Buss KA. Moderators of the relation between shyness and behavior with peers: Cortisol dysregulation and maternal emotion socialization. Social Development. 2012; 21(4):801–820. [PubMed: 23226925]

Degnan KA, Henderson HA, Fox NA, Rubin KH. Predicting social wariness in middle childhood: The moderating roles of childcare history, maternal personality and maternal behavior. Social Development. 2008; 17(3):471–487. [PubMed: 20463856]

Dollar JM, Stifter CA. Temperamental surgency and emotion regulation as predictors of childhood social competence. Journal of Experimental Child Psychology. 2012; 112(2):178–194. [PubMed: 22414737]

Donzella B, Gunnar MR, Krueger WK, Alwin J. Cortisol and vagal tone responses to competitive challenge in preschoolers: Associations with temperament. Developmental Psychobiology. 2000; 37(4):209–220. [PubMed: 11084602]

Dougherty LR, Klein DN, Rose S, Laptook RS. Hypothalamic-pituitary-adrenal axis reactivity in the preschool-age offspring of depressed parents: Moderation by early parenting. Psychological Science. 2011; 22(5):650–658. [PubMed: 21460339]

Edwards SL, Rapee RM, Kennedy S. Prediction of anxiety symptoms in preschool aged children: Examination of maternal and paternal perspectives. Journal of Child Psychology and Psychiatry. 2010; 51:313–321. [PubMed: 19769584]

Graham JW. Missing data analysis: Making it work in the real world. Annual Review of Psychology. 2009; 60:549–576.

Goldsmith HH, Buss AH, Plomin R, Rothbart MK, Thomas A, Chess S, McCall RB. Roundtable: What is temperament? Four approaches. Child Development. 1987; 58:505–529. [PubMed: 3829791]

Goldsmith, HH.; Rothbart, MK. The laboratory temperament assessment battery (locomotor version 3.1). Madison, WI: The University of Wisconsin-Madison; 1999.

Gunnar, MR. The role of glucocorticoids in anxiety disorders: A critical analysis. In: Vasey, MW.; Dadds, MR., editors. Developmental psychopathology of anxiety. New York: Oxford University Press; 2001. p. 143-159.

Gunnar MR, Morrison SJ, Chisholm K, Schuder M. Salivary cortisol levels in children adopted from Romanian orphanages. Development and Psychopathology. 2001; 13(3):611–628. [PubMed: 11523851]

Gunnar M, Quevedo K. The neurobiology of stress and development. Annual Review Psychology. 2007; 58:145–173.

Gunnar MR, Talge NM, Herrera A. Stressor paradigms in developmental studies: What does and does not work to produce mean increases in salivary cortisol. Psychoneuroendocrinology. 2009; 34(7): 953–967. [PubMed: 19321267]

Gunnar MR, Tout K, de Haan M, Pierce S, Stansbury K. Temperament, social competence, and adrenocortical activity in preschoolers. Developmental Psychobiology. 1997; 31(1):65–85. [PubMed: 9222117]

Hastings PD, Ruttle PL, Serbin LA, Mills RS, Stack DM, Schwartzman AE. Adrenocortical responses to strangers in preschoolers: Relations with parenting, temperament, and psychopathology. Developmental Psychobiology. 2011; 53(7):694–710. [PubMed: 21432849]

Hill NE, Bush KR. Relationships between parenting environment and children's mental health among African American and European American mothers and children. Journal of Marriage and the Family. 2001; 63:654–966.

Jansen J, Beijers R, Riksen-Walraven M, de Weerth C. Cortisol reactivity in young infants. Psychoneuroendocrinology. 2010; 35(3):329–338. [PubMed: 19651476]

Kagan, J. The concept of behavioral inhibition to the unfamiliar. In: Reznick, JS., editor. Perspectives on behavioral inhibition. Chicago, IL: The University of Chicago Press; 1989. p. 1-23.

Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioral inhibition in children. Child Development. 1987; 58:1459–1473. [PubMed: 3691195]

Kagan, J.; Snidman, N.; Arcus, D.; Reznick, JS. Galen's prophecy: Temperament in human nature. New York, NY: Basic Books; 1994. p. 170-207.

Kiel EJ, Buss KA. Prospective relations among fearful temperament, protective parenting, and social withdrawal: The role of maternal accuracy in a moderated mediation framework. Journal of Abnormal Child Psychology. 2011; 39(7):953–966. [PubMed: 21537895]

Kiel EJ, Buss KA. Associations among context-specific maternal protective behavior, toddlers' fearful temperament, and maternal accuracy and goals. Social Development. 2012; 21(4):742–760. [PubMed: 23226924]

Nachmias M, Gunnar M, Mangelsdorf S, Parritz RH, Buss K. Behavioral inhibition and stress reactivity: The moderating role of attachment security. Child Development. 1996; 67(2):508–522. [PubMed: 8625725]

McEwen BS. Stress, adaptation, and disease: Allostasis and allostatic load. Annals of the New York Academy of Sciences. 1998; 840(1):33–44. [PubMed: 9629234]

McEwen BS, Stellar E. Stress and the individual: Mechanisms leading to disease. Archives of Internal Medicine. 1993; 153(18):2093–2101. [PubMed: 8379800]

Mount KS, Crockenberg SC, Jó PSB, Wagar JL. Maternal and child correlates of anxiety in 2½-year-old children. Infant Behavior and Development. 2010; 33(4):567–578. [PubMed: 20723999]

Rappolt-Schlichtmann G, Willett JB, Ayoub CC, Lindsley R, Hulette AC, Fischer KW. Poverty, relationship conflict, and the regulation of cortisol in small and large group contexts at child care. Mind, Brain, and Education. 2009; 3(3):131–142.

Rubin KH, Burgess KB, Hastings PD. Stability and social–behavioral consequences of toddlers' inhibited temperament and parenting behaviors. Child Development. 2002; 73(2):483–495. [PubMed: 11949904]

Rubin KH, Cheah CSL, Fox N. Emotion regulation, parenting, and display of social reticence in preschoolers. Early Education and Development. 2001; 12:97–115.

Rubin KH, Hastings PD, Stewart SL, Henderson HA, Chen X. The consistency and concomitants of inhibition: Some of the children, all of the time. Child Development. 1997; 68(3):467–483. [PubMed: 9249961]

Shirtcliff EA, Peres JC, Dismukes AR, Lee Y, Phan JM. Hormones: Commentary: Riding the physiological roller coaster: Adaptive significance of cortisol stress reactivity to social contexts. Journal of Personality Disorders. 2013; 28(1):40–51. [PubMed: 24344886]

Talge NM, Donzella B, Gunnar MR. Fearful temperament and stress reactivity among preschool-aged children. Infant and Child Development. 2008; 17(4):427–445. [PubMed: 19122850]

Thompson, RA. Childhood anxiety disorders from the perspective of emotion regulation and attachment. In: Vasey, MW.; Dadds, MR., editors. The developmental psychopathology of anxiety. New York, NY: Oxford University Press; 2001. p. 160-182.

Ursache A, Blair C, Granger DA, Stifter C, Voegtline K. Behavioral reactivity to emotion challenge is associated with cortisol reactivity and regulation at 7, 15, and 24 months of age. Developmental Psychobiology. 2013; 56(3):474–488. [PubMed: 23918193]

Highlights

- Toddler cortisol reactivity has previously been linked to the emotion of fear.
- Mothers' spontaneous comforting behavior related to cortisol reactivity to a fear.
- Low-threat fear moderated the relation between cortisol reactivity and anxiety.
- High-threat fear did not moderate the relation between cortisol reactivity and anxiety.

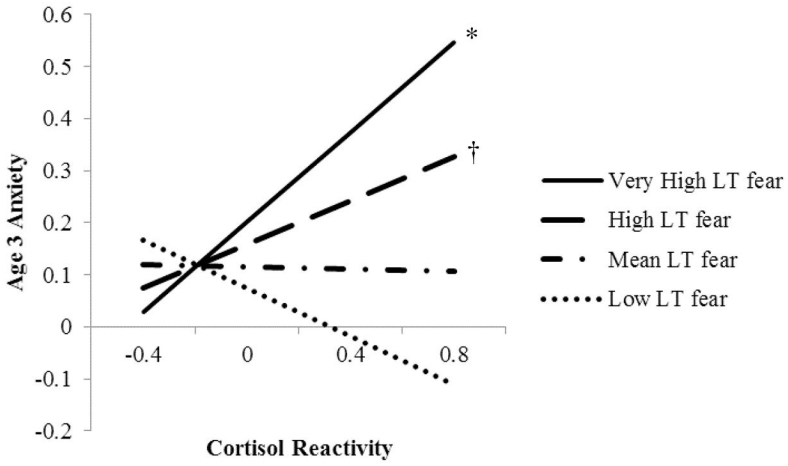

Figure 1.

Interaction between toddler cortisol reactivity and fear expressed in a low-threat (LT) context in predicting anxiety. Toddler cortisol reactivity significantly predicted age 3 anxiety only at very high (+2 SD) of low-threat fear. The regression model controlled for SES and age 2 anxiety, so the model predicted change in anxiety over a 1 year period. $^{\dagger}p < .10$, $^{*}p < .05$.

Table 1

Descriptive Statistics and Bivariate Correlations

Variable	*Mean*	*SD*	*Range*	2	3	4	5	6	7
1. Cortisol reactivity	0.00	0.20	−0.46 – 0.82	.31**	.02	.00	.21*	.02	.04
2. High-threat fear	2.96	1.18	1.00 – 5.00	--	.25*	−.11	.24*	−.02	.08
3. Low-threat fear	2.10	0.90	1.00 – 4.50		--	.38***	.37***	.10	.21*
4. Solicited comforting	0.09	0.20	0.00 – 1.17			--	.13	.16	.07
5. Unsolicited comforting	0.06	0.15	0.00 – 1.04				--	−.03	−.05
6. Age 2 anxiety	0.46	0.23	0.00 – 1.09					--	.45***
7. Age 3 anxiety	0.41	0.17	0.00 – 0.91						--

Note. Cortisol reactivity represents the unstandardized residual from a regression equation in which baseline cortisol secretion predicted post-visit cortisol secretion. Statistics are provided for Age 2 anxiety and Age 3 anxiety after imputation of missing values.

* $p < .05$,

** $p < .01$,

*** $p < .001$

Table 2

Regression Model Predicting Cortisol Reactivity from Toddler Fear and Maternal Comforting Behavior

Variable	*b* (SE)	β	*t*-value	*p*-value	95% CI	sr^2
Intercept	0.01 (0.05)	--	0.16	.876	−0.10, 0.11	--
Low-threat fear	−0.01 (0.03)	−0.06	−0.50	.619	−0.07, 0.04	.003
Solicited comforting	−0.01 (0.11)	−0.01	−0.06	.956	−0.23, 0.22	.000
Unsolicited comforting	0.30 (0.14)	0.23	2.11	.037	0.02, 0.59	.045

Note. The overall regression model was not significant (R^2 = .05, F[3,95] = 1.51, p = .218). CI = confidence interval. sr^2 = squared semi-partial correlation.

Table 3

Regression Models Predicting Age 3 Anxiety

Variable	*b* (SE)	β	*t*-value	*p*-value	95% CI	sr^2
Low-Threat Fear Model						
Intercept	0.12 (0.09)	--	1.29	.215	−0.07, 0.30	--
SES	0.00 (0.00)	0.17	1.67	.099	0.00, 0.01	.022
Age 2 Anxiety	0.36 (0.08)	0.47	4.80	< .001	0.21, 0.51	.179
Cortisol reactivity	−0.01 (0.09)	0.01	−0.12	.902	−0.18, 0.88	.000
Fear	0.05 (0.02)	0.24	2.32	.022	0.01, 0.09	.042
Solicited comfort	−0.11 (0.09)	−0.12	−1.18	.240	−0.28, 0.07	.011
Unsolicited comfort	−0.24 (0.14)	−0.21	−1.74	.085	−0.52, 0.03	.024
Cortisol reactivity X Fear	0.25 (0.10)	0.27	2.44	.017	0.05, 0.44	.046
Cortisol reactivity X Solicited comfort	−0.86 (0.91)	−0.11	−0.94	.348	−2.67, 0.95	.007
Cortisol reactivity X Unsolicited comfort	0.01 (0.36)	0.00	0.02	.986	−0.70, 0.71	.000
High-Threat Fear Model						
Intercept	0.15 (0.09)	--	1.75	.084	−0.02, 0.32	--
SES	0.00 (0.00)	0.11	1.12	.264	−0.00, 0.00	.010
Age 2 Anxiety	0.37 (0.07)	0.48	4.95	< .001	0.22, 0.51	.200
Cortisol reactivity	−0.07 (0.09)	−0.08	−0.71	.481	−0.25, 0.12	.004
Fear	0.01 (0.01)	0.09	0.91	.366	−0.02, 0.04	.007
Cortisol reactivity X Fear	0.12 (0.08)	0.17	1.56	.122	−0.03, 0.28	.020

Note. The low-threat fear model was significant (R^2 = .31, F[9,89] = 4.41, p < .001), as was the model for high-threat fear (R^2 = .24, F[5,93] = 5.90, p < .001). CI = confidence interval. sr^2 = squared semi-partial correlation.