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Caregiver Protective Behavior, Toddler Fear and Sadness, and Toddler Cortisol Reactivity in Novel Contexts

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Abstract

Previous research has shown that caregiver protective behavior may exacerbate toddler distress in specific contexts. The current study sought to extend this work to examine associations between these variables and toddler cortisol reactivity. Ninety-three 24-month-old toddlers were observed across six novel contexts designed to elicit distress. Toddlers were asked to give saliva samples at the beginning and end of the laboratory procedure. Toddler sadness, toddler fear, and caregiver protective behavior were coded. Results indicate that caregiver protective behavior accounted for the association between toddler sadness and cortisol reactivity where higher levels of protective behavior were associated with higher cortisol reactivity. The current study showed that caregiver protective behavior, which functions to prevent a child from interacting with a novel stimulus, is an important mechanism to consider when understanding toddler stress responses during novel contexts.

Keywords

parenting; protectiveness; toddlers; stress reactivity; cortisol; sadness

From even before birth, child physiological regulation is guided and influenced by parents, and this influence continues well into childhood (e.g., Gunnar & Donzella, 2002). One important physiological system that has been linked to parent behavior is the stress-response system, specifically the hypothalamic-pituitary-adrenocortical (HPA) axis. Within this system, psychological stress can trigger physiological responses (Gunnar & Quevedo, 2007). These physiological responses can be measured by glucocorticoids, hormones secreted by the HPA axis in response to psychological stress. Research has found that certain types of early childhood stress are related to elevated levels of cortisol, a type of glucocorticoid secreted in humans (Gunnar & Quevedo, 2007).

One type of normative stress for children includes encountering novel experiences and situations. Research has shown that children react to these types of experiences with both behavioral indicators (e.g., freezing, crying, avoiding) and physiological indicators of stress (increased cortisol, increased autonomic activity) (e.g. Buss, Davidson, Kalin, & Goldsmith, 2004; Fortunato, Dribin, Granger, & Buss, 2008). Although these direct effects on child

stress responses are observed, these effects are often qualified by other factors such as context, temperament, and parenting behavior. Specifically, caregiver behavior has been shown to buffer or exacerbate child stress responses in novel situations (Coplan, Arbeau, & Armer, 2008; Gunnar, 1990) such that ineffective caregiving strategies have been shown to be associated with increased stress responses in children (Gunnar, Larson, Hertsgaard, Harris, & Brodersen, 1992). One type of ineffective caregiving strategy may be overprotective behavior, in which parents prevent their children from engaging with stimuli in the environment. It is possible that overprotective parenting results in increased stress for children. The current study examines the role of caregiver behavior in the association between child factors (such as emotion expression and behavior) and child cortisol reactivity.

The HPA Axis and the Stress-Response System

The HPA axis is a part of the stress-response system that maintains a homeostatic balance in the body (Fisher & Stoolmiller, 2008). Generally, cortisol acts to sustain physiological systems in working balance as they act in response to normal changes in functioning (Fox, Hane, & Perez-Edgar, 2006). As the end product of the HPA axis response, high levels of cortisol have been shown to produce negative effects on the body, such as impairments in neural plasticity, memory, and learning; inhibitions in glucose utilization in the brain; and activities that lead to the blunting of the HPA response to stress (Gunnar & Quevedo, 2007). The association between cortisol levels and functioning typically reflects an inverted-U shaped curve such that very low levels as well as very high levels of cortisol are associated with impairments in functioning, whereas moderate levels of cortisol are associated with better physical and mental health (Sapolsky, 1997).

The HPA axis has been highlighted as a mechanism through which to understand the influence of external stressors on children's physiological functioning. Davies, Sturge-Apple, Cicchetti, and Cummings (2007) posited that exposure to chronic environmental stress may sensitize the HPA axis to subsequent stressors. Thus, the more exposure to environmental stress a child experiences, the more hyperactivity of the HPA system in anticipating external stress occurs. This process presumably results in the sensitization of the child's emotional and behavioral responses and cortisol reactivity. Although this process has been shown to occur for children in adverse environments, children in less adverse environments have also been shown to be susceptible to increased activity in the HPA axis as a result of external stress (see reviews by Gunnar & Donzella, 2002; Gunnar & Quevedo, 2007). Children displaying different temperamental traits may be more susceptible to the effects of these types of environmental stressors than other children may (e.g., Buss, Davis, & Kiel, 2011; Essex, Armstrong, Burk, Goldsmith, & Boyce, 2011).

Child Factors Contributing to Stress Response

Temperament refers to individual differences in reactivity and self-regulation processes that moderate reactivity and that are influenced by an individual's biological makeup (Rothbart, 2007). Research has shown that different types of temperament and behaviors reflecting temperamental traits are associated with cortisol levels. Behavioral inhibition and fearful behavior more broadly have been shown to relate to higher baseline cortisol levels (Buss et al., 2004; Fox, Henderson, Marshall, Nichols, & Ghera, 2005; Kagan, Reznick, & Snidman, 1987). Likewise, child internalizing behavior is also associated with high baseline levels of cortisol and lower reactivity in stress-related physiological systems during toddlerhood and preschool ages (Granger, Stansbury, & Henker, 1994; Granger, Weisz, Ikeda, McCracken, & Douglas, 1996; Kagan, Reznick, Snidman, 1987; Smider et al., 2002). Moreover, the association between internalizing behavior and cortisol levels has been found to be relatively

stable, as children rated as withdrawn by their teachers showed higher baseline levels of cortisol a year and a half earlier at the age of 4.5 years (Smider et al., 2002). Toddlers' displays of negative affect and withdrawal, as well as freezing behavior in specific types of social contexts, were also related to increased cortisol reactivity for children following a stressful situation (Buss et al., 2004; Fortunato et al., 2008). In addition, toddlers with more sophisticated mental development (as measured from the Bayley Scales of Infant Development) and higher levels of observer-rated attentional abilities had a cortisol response to a challenge suggestive of self regulation, a pattern characterized by an initial increase followed by decrease in cortisol (i.e., recovery) (Blair et. al, 2008). In general, research has shown that rhythms reflecting low baseline cortisol and increases in cortisol, followed by decreases, have been associated with cognitive and socioemotional competence (Blair, Granger, & Razza, 2005; Davis, Bruce, & Gunnar, 2002). Thus, variability in the magnitude of cortisol reactivity is an important indicator of physiological distress.

In addition to temperamental traits and related behaviors, research has highlighted associations between the displays of certain emotions in children and elevations in cortisol reactivity. Distress, specifically sadness, in response to external stimuli appears to be linked to elevations in cortisol reactivity relative to baseline in infants (Lewis & Ramsay, 2005; Lewis, Ramsay, & Sullivan, 2006). Moreover, the expression and elicitation of sadness, and not the recovery of sadness appeared responsible for the increase in cortisol (Lewis & Ramsay, 2005). In addition to sadness, child fear has been linked to increases in cortisol (Buss et al., 2004), particularly in situations with insensitive caregiving or insecure attachment relationships (Gunnar & Donzella, 2002; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996). However, several studies failed to replicate findings of an association between fearful behavior and cortisol reactivity (e.g., Buss, Schumacher, Dolski, Kalin, Goldsmith, & Davidson, 2003; Kagan, Reznick, Snidman, Gibbons, & Johnson, 1988; Schmidt, Fox, Schulkin, & Gold 1999)). Thus, the associations among types of distress responses, eliciting conditions, and resulting changes in cortisol warrant continued exploration. It is unclear how these emotions come to be associated with cortisol responses, but one possible mechanism may be the caregiving environment.

Caregiver Contributions to Child Stress Response

Early environmental experiences, specifically those involving social experiences with caregivers, have been shown to contribute to the responsivity and regulation of the stress-response system (Gunnar & Donzella, 2002; Levine, 1994). Disruptions in the parent-child relationship and unsupportive parent-child interactions have been shown to relate to increased levels of baseline cortisol during preschool, and behavioral and emotional difficulties later (Essex, Klein, Cho, & Kalin, 2002). Moreover, parents' own stress levels have been shown to relate to child HPA functioning (Tu et al., 2007). Mothers who reported higher stress levels and showed more interactive behavior had infants whose higher baseline cortisol was associated with poorer focused attention (Tu et al., 2007). When parents are stressed themselves, their ability to be sensitive and use effective strategies with children to promote positive development is hindered (Halme, Tarkka, Nummi, & Astedt-Kurki, 2006).

Although much of the research on cortisol reactivity and parenting focuses on children in harsh environments, children in low risk environments also appear to be affected by these types of parenting behaviors. Cortisol patterns over a typical day were associated with attention and stimulation from caregivers in home-based childcare (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000). Children who received relatively low levels of attention from childcare providers compared to children who received higher levels of attention displayed an atypical pattern of cortisol levels over the course of the day (i.e., an increase from morning to afternoon), suggesting that even a normative caregiving response (occasional

inattention) influences cortisol in children. Moreover, during a situation in which an infant was left with a substitute caregiver instead of his or her mother, infant cortisol increased relative to baseline when these caregivers only responded to infant distress and did not display warmth and continuous support throughout the interaction (Gunnar et al., 1992). If evidence suggests that a childcare provider can affect a child's cortisol levels over the course of one day or one interaction, it is likely that parents have an even larger influence on child cortisol levels on a daily basis. It has been posited that when parents use attentive caregiving they encourage children and allow them to express and communicate distress in constructive ways without resulting in increases in cortisol (Gunnar & Donzella, 2002).

Thus, it appears that certain types of parenting behaviors in particular contexts increase child stress responses. When parents encourage children with attentive and responsive parenting, child distress behavior decreases (Dadds & Roth, 2001). However, parenting behaviors that restrict a child's behavior, such as overprotective behavior, may be associated with increases in child distress. Past research has discussed overprotective behavior as the encouragement of dependency on parents, intrusion, and controlling children's behavior resulting in the lack of development as an individual (Parker, Tupling, & Brown, 1979). More recently, it has been described as a component of psychological control whereby parents restrict children's autonomy (Barber, Olsen, & Shagle, 1994; Mills & Rubin, 1998). Even in less severe cases, protective behavior may be thought of as discouragement of exploration of unfamiliar stimuli, restriction of activities, and intrusion on activities (Rubin, Burgess, & Hastings, 2002). It is thought that these behaviors (even in small quantities) may prevent children from learning coping strategies and becoming autonomous, and instead result in stress from their caregiver's stress response cues.

Consistent with this idea, Buss and Kiel (2011) discovered that relatively high levels of caregiver protective behavior were associated with the maintenance of toddler behavioral distress for toddlers with high levels of distress. The authors posited that this type of maternal behavior might represent overprotection and possibly undermine the child's own coping strategies when it precedes child distress. They further suggest that too much protective behavior may actually be aversive for children, and consistent with findings that parenting behavior results in a behavioral distress response, it is also possible that it results in a physiological stress response (such as cortisol reactivity), particularly for certain children. Thus, caregiver overprotective behavior, although undertaken with the goal of soothing distress, may function to restrict the child's autonomy as well as reinforce distress.

Caregiver Overprotection, Child Temperament, and Child Stress Response

In addition to the influence on child stress responses, caregiver overprotection also has effects on child behavior and temperamental responses. Rubin et al. (2002) found that a parenting style reflecting overprotective behavior moderated the association between shyness and behavioral inhibition at 2 years of age, such that toddler shyness and behavioral inhibition were only related for toddlers of mothers with high levels of intrusive control. Maternal overprotective behavior has also been found to moderate relations between shyness and child maladjustment in kindergarten (Coplan et al., 2008). Moreover, across development, it has been shown that maternal overprotective behavior was associated with a sustained trajectory of withdrawal/fearfulness (e.g., Mount, Crockenberg, Bárrig Jó & Wagar, 2010; Rubin et al., 2002; Rubin, Coplan, & Bowker, 2009). In addition to moderation, Kiel and Buss (2010; 2011) showed that protective behavior mediates the relation from fearful temperament to later shyness/inhibition. It has been found recently that this mediation may be context-specific. Consistent with this idea, Kiel and Buss, in press discovered that in low-threat novel contexts, as opposed to high-threat novel contexts, maternal overprotective behavior mediated the relation between fearful temperament and

later shyness/inhibition. Thus, caregiving behavior appears to affect the child behaviors associated with fearful temperament.

Evidence has begun to show that these overprotective behaviors do not simply buffer child fear responses, but may be responsible for them independent of child factors (e.g., Kiel & Buss, in press). When considering evidence that caregiver protective behaviors have been linked directly to child distress and child cortisol (e.g., Gunnar & Donzella, 2002; Rubin et al., 2002), as well as emerging evidence on the mediation of the relation from child temperamental traits to child outcomes by caregiver overprotective behavior (Kiel & Buss, in press), the role of caregiver protective behavior in the relation between child distress and cortisol reactivity warrants further exploration.

The current study extended the findings from Buss and Kiel (2011) and Kiel and Buss (2011; in press) on caregiver overprotective behavior and toddler distress to additionally examine the associations between these variables and toddler cortisol reactivity. Children who are exposed to environmental stress (in the form of overprotection parenting) may show relatively high levels of behavioral distress and increased cortisol reactivity relative to baseline during stressful situations.

The Current Study

In the current study, we observed 93 24-month-old toddlers across six novel contexts designed to elicit distress in toddlers. Toddlers were asked to give saliva samples at the beginning and end of the laboratory procedure. Toddler sadness, toddler fear, and caregiver protective behavior were coded. Consistent with evidence suggesting that ineffective caregiving relates to increased cortisol reactivity in certain contexts (Gunnar & Donzella, 2002) and that caregiver overprotective behavior is associated with increased behavioral distress in children (Buss & Kiel, 2011), it is thought that high levels of caregiver protective behavior would not only be associated with increased behavioral distress, but would also be associated with increased cortisol reactivity following a stressor.

Moreover, given evidence that maternal protective behavior was found to occur prior to child behavioral distress, suggesting that it contributed to the maintenance of distress (Buss & Kiel, 2011), it was hypothesized that caregiver protective behavior would account for the relation between child distress and cortisol reactivity. Child sadness and fear were examined separately as two types of distress. They are considered discrete emotions that have different functions to organize behavior (Izard & Ackerman, 2000). Moreover, little research exists on the associations between sadness and cortisol reactivity, and contradictory research exists on the associations between fear on cortisol reactivity (e.g., Buss et al., 2003; Nachmias et al., 1996; Schmidt et al., 1999). Thus, we wanted to examine sadness separately from fear to disentangle types of toddler distress and associations with cortisol and caregiver protective behavior.

Methods

Ninety-three toddlers (24 months; 56.3 % male) and their mothers participated in an ongoing study of emotion development. Participants were predominantly Caucasian (92% Caucasian; 3.6% African-American; 1.8% Hispanic; 1.8% Asian-American; 0.9% American-Indian) and income ranges were predominantly in the middle class range (7.3% in the lower SES bracket below \$30,000 and 50% above \$60,000). Approximately two-thirds of the children spent some time during the week in non-maternal care (e.g., daycare or preschool), for an average of 4 days/week.

Mothers were told that their children would be participating in several different tasks designed to elicit reactions to novel people and objects. Episodes alternated between two rooms separated by a control room from which videotaping took place through one-way mirrors. Two cortisol samples were taken from children: one at the beginning and one at the end of the laboratory procedure (one prior to the first episode and one following the final episode). The laboratory procedure was completed during various times of the day depending on family availability (varying from morning to evening), with the average time being 11:30am for the first sample and 1:00pm for the second sample. Twenty-seven toddlers were missing either pre-visit or post-visit cortisol data due to refusal or insufficient amounts of saliva within samples, and the current sample required both measures. Missing data for salivary cortisol is typical at this age (e.g., Nachmias et al., 1996). Thus, the total sample for the analyses included 66 mother-toddler pairs. Toddlers missing data were compared with toddlers with cortisol data, and there were no significant differences in demographic variables or variables of interest.

Procedure

The current study focused on a series of six novel tasks designed to elicit individual differences in distress to novelty. This paradigm is consistent with that used in previous studies (see Buss, 2011) and is a modification of the toddler and preschool versions of the Laboratory Temperament Assessment Battery, Lab-TAB (Buss & Goldsmith, 2000; Goldsmith, Reilly, Lemery, Longley, & Prescott, 1994). Order effects have not been observed previously, nonetheless toddler participated in 1 in 4 partially counterbalanced episode orders (as described in Buss, 2011) to control for the possibility of order effects. Previous research using this paradigm has not found effects of episode order on general emotionality or specific behaviors (e.g., Buss, 2011; Kiel & Buss, 2010).

Three of the tasks consisted of toddlers' interactions with an unfamiliar adult. *Stranger Approach* consisted of a short conversation with a male stranger while the toddler played with toys, *Stranger Working* involved an unfamiliar female stranger completing paperwork in the corner without initiating any interaction with the child, and *Clown* consisted of a female clown who asked the toddler to play three different activities with her. All of these episodes lasted approximately three minutes. The three other tasks included toddlers' interaction with a novel object. *Puppet Show* lasted approximately three minutes and involved elephant and lion puppets that invited the toddler to play catch, go fish, and take a sticker. The child was invited to touch the puppets after the show was over. In the *Robot* episode, the experimenter brought the caregiver and child into the room and had the child sit on the caregiver's lap in the corner to standardize baseline behavior so individual differences in behavior across the episodes were more meaningful. A remote-controlled robot, controlled from behind the one-way mirror, moved around for one minute. The experimenter then came back into the room and asked the child to touch the robot with three prompts before ending the episode. During the *Spider* episode, the experimenter brought the caregiver and child into a room and also instructed the toddler to sit in the caregiver's lap in the corner. A large stuffed animal spider attached to a remote control car was placed in the opposite corner prior to the child's entrance. The spider, controlled by a remote through the one-way mirror, moved halfway towards the pair, paused for 10 seconds, and then retreated back to the corner. It then moved all the way towards the pair, remained for 10 seconds, and then retreated back to the corner. The experimenter then entered and asked to child to touch the spider with three prompts before ending the episode. This was the final episode prior to the second sample of saliva collection.

Behavioral Assessment

Toddler distress—Several behaviors were scored on a second-by-second basis for the duration of each episode. Specifically, toddlers' facial and bodily fear and sadness were scored every time they occurred according to the AFFEX coding system (Izard, Dougherty, & Hembree, 1983). This system requires coders to focus on three regions of the face and rate affect on a 0 to 3 intensity scale that summarizes muscle movement across the face. A score of "0" indicated the absence of an expression. A score of "1" indicated a fleeting or moderate expression in one facial region. A score of "2" indicated moderate expressions in 2 facial regions or a strong emotion expression in one facial region. A score of "3" indicated a strong emotion expression in at least 2 facial regions. Facial fear reflected raised and drawn together eyebrows, raised or tense eyelids, and and/or an open mouth with the corners pulled straight back. Facial sadness reflected raised outer eyebrows, narrow or squinted eyes, and/or the mouth corners pulled out and down, often resulting in upper lip protrusion. Bodily expressions and crying were scored on a similar 0 to 3 intensity scale, with 3 reflecting the maximum intensity. Bodily fear reflected reduced activity, tensing of the muscles, trembling, or freezing. Bodily sadness reflected slumping or dropping of the head. Crying ranged from whimpering to full intensity crying or screaming.

A composite was formed across all six episodes for sadness from facial and bodily sadness, and for fear from facial and bodily fear, based on all of the ratings at each second for each episode. Undergraduate coders were trained to a reliability of 90% agreement, $\kappa = .65$, and reliability was performed for 20% of all cases double coded across episodes. Percentage agreement ranged from .88–.91 and Kappa ranged from $\kappa = .50$ to .89¹. Behaviors during these episodes were entered into an exploratory principle component extraction, resulting in distinct fear and sadness composites across all episodes (see Buss, 2011). Most composites were positively correlated across episodes ($r = .02$ through .58, average of $r = .23$) and the current study sought to examine typical behavior, so composites for toddler sadness and fear were averaged across episodes to create an overall sadness and fear composite. Any outliers were truncated (i.e., to be within three standard deviations of the mean), resulting in an acceptable level of skewness (Fear Skewness = .53; Sadness Skewness = 1.71).

Caregiver protective behavior—Caregivers were scored for the intensity of protective behaviors in each episode. Protective behavior in the current study consisted of a caregiver shielding or blocking his or her child from the stimulus, including pulling the child into his or her lap. Coders rated the intensity of this behavior in 10-second epochs across all episodes. The behaviors were scored on a scale from 0–3 with 0 = no behavior shown, 1 = slight behavior shown (e.g. leaning the child away from the stimulus or putting arm in front of child), 2 = moderate behavior shown (e.g. picking up the child or moving him away from the stimulus), 3 = intense and prolonged "2" behavior shown (e.g. turning the child completely away from the stimulus or asking to end the episode). Percent-agreement reliability averaged .96 across episodes. A composite was created based on the average mean and maximum level of protective behavior across all six episodes. Given the skewed nature of the level of protectiveness (several mothers showed no protective behavior), protective behavior was transformed using a square root transformation, and outliers were truncated (Skewness = 2.35).

Saliva Collection

Children were asked not to consume food or drink 30 minutes prior to giving saliva samples. Samples were collected using cotton swabs, and children were allowed to mouth the swab

¹When scoring second-by-second many cells result in zeros which unbalances the matrix used to calculate kappa and thus artificially lowers them. See Buss (2011) for more details on reliability calculation for this sample.

after a small amount of sugar crystals were put on the end of the swab to encourage compliance. Although certain stimulants have been found to lower pH levels and increase cortisol levels, prior work using the same methodology to collect toddler saliva has shown that there is no difference in pH levels with the use of sugar crystals compared with no substances, suggesting that these stimulants would not influence the data (Buss et al., 2004). After the visit, samples were kept frozen until they were analyzed. The samples were then transported to the Behavioral Endocrinology Lab at Penn State University and analyzed. Samples were assayed for salivary cortisol using an enzyme immunoassay US FDA (510) cleared for use as an in vitro diagnostic measure of adrenal functioning (Salimetrics, State College, PA). The test used 25 μ L of saliva, ranged in sensitivity from .007 to 3.0 μ g/dL, and had an average intra-and inter-assay CV of less than 5% and 10%, respectively.

Cortisol variables were examined for statistical outliers ($<$ or $>$ 3 SDs), which were recoded to the next highest value in the respective distribution. The percent change in cortisol from the first sample prior to the visit to the post sample following the visit was calculated to reflect cortisol reactivity using a standard formula $\{(\text{postbaseline cortisol} - \text{prebaseline cortisol}) / \text{prebaseline cortisol}\}$ as used in previous studies (e.g., Goldberg et al., 2003; Luby, Heffelfinger, Mrakotsky, Brown, Hessler, & Spitznagel, 2003). Thus, a value of 1 would suggest that postbaseline value increased by 100% of the prebaseline value, or doubled from prebaseline. Means and standard deviations of all raw variables are shown in Table 1. As in previous studies (e.g., Fortunado et al., 2008; Scarpa & Luscher, 2002), percent change was chosen to reflect the change in cortisol in response to the tasks because it allows for interpretation of reactivity regardless of the initial mean values of cortisol, whereas difference scores can reflect, in part, baseline levels (i.e., law of initial values).

Bivariate correlations between cortisol samples and time of day were not significant ($r = .01$, and $-.01$ for pre- and post- laboratory samples, respectively). Moreover, the time between the pre- and post- samples was not significantly associated with percent change in cortisol ($r = -.16$). However, to account for possible effects of time of day on cortisol reactivity, time of the pre-sample cortisol measure was regressed onto cortisol reactivity, and the standardized residual was used in the following regression model for cortisol reactivity.

Results

Bivariate correlation analyses were performed among the variables of interest (child sadness, child fear, caregiver protective behavior, and cortisol reactivity) (see Table 2). Toddler sadness was positively correlated with toddler fear, caregiver protective behavior, and cortisol reactivity. A scatterplot displaying the association between the final cortisol reactivity variable and child sadness is presented in Figure 1. Toddler fear was positively correlated with caregiver protective behavior but was not significantly associated with cortisol reactivity. Caregiver protective behavior was positively correlated with cortisol reactivity.

Due to the lack of significant correlation between toddler fear and cortisol reactivity, toddler fear was not examined in a mediation model. Mediation for the sadness model was tested by examining traditional paths of mediation as well as by using the Sobel test (Baron & Kenny, 1986) and the distribution of products method (MacKinnon, Lockwood, Hoffman, West, & Sheets, 2002), which provides a direct test of the indirect effect of the association between toddler sadness and cortisol reactivity through caregiver protective behavior. Figure 2 shows the regression coefficients for each path in the current mediation model. Higher levels of toddler sadness significantly predicted caregiver protective behavior and cortisol reactivity, and higher levels of caregiver protective behavior significantly predicted higher levels of cortisol reactivity above and beyond toddler sadness. Moreover, the association between

toddler sadness and cortisol reactivity was no longer significant when considering the influence of caregiver protective behavior. Results of the Sobel test indicated that caregiver protective behavior was a mediator in explaining the association between toddler sadness and cortisol reactivity (Sobel = 2.51, $p < .05$).

However, Sobel's test may be inaccurate due to violations of the assumption that the indirect effect follows a normal distribution, which are particularly likely to occur in small samples. The distribution of product method is an alternative approach that has been found to be more accurate in testing the significance of the indirect effect (MacKinnon et al., 2002). Instead of assuming a normal distribution, this technique determines the actual distribution of the indirect effect and derives critical values from which a confidence interval can be formed. Therefore, we used this approach as an alternative test of the indirect effect. Using the PRODCLIN macro for SPSS (MacKinnon, Fritz, Williams, & Lockwood, 2007), we found that the confidence interval for the indirect effect provided further evidence for its significance (95% CI [0.004, 0.04]).

In order to be confident in our hypothesis, we tested several alternate models of the data. First, we tested caregiver protective behavior as a moderator rather than a mediator. This interaction was not significant ($\beta = .15$, $p = ns$). Second, we tested sadness as the mediator of the relation between caregiver protective behavior and toddler cortisol reactivity. However, this model could not be tested because the path from sadness to cortisol reactivity was not significant when caregiver protective behavior was in the model (as seen in Figure 2). Finally, we tested protective behavior as a moderator on the association between fear and cortisol reactivity, and the interaction was not significant ($\beta = .61$, $p = ns$). Therefore, the model with protective behavior as a mediator appears to be the best reflection of the data.

Discussion

This study examined the contributions of toddler distress and caregiver protective behavior to toddler cortisol reactivity. Results indicate that caregiver protective behavior accounted for the association between toddler sadness and cortisol reactivity. Consistent with previous studies, toddler emotion expression was associated with cortisol reactivity. Sadness has been shown to be a discriminating emotion in predicting cortisol reactivity compared with other emotions (e.g., Lewis & Ramsay, 2005). Accordingly, whereas sadness did relate, toddler fear was not associated with cortisol reactivity. Previous research has resulted in contradictory evidence for the association between fear and cortisol reactivity. Consistent with the current study, some studies have found significant associations between fear and cortisol and others have not found direct associations (e.g., Buss et al., 2004; Buss et al., 2003; Gunnar & Donzella, 2002; Kagan et al., 1988; Schmidt et al., 1999). One possible explanation for these inconsistent findings is that other factors interact with fear to predict cortisol reactivity. For example, Nachmias et al. (1996) found that attachment security moderated the association between fearful behavior and cortisol reactivity. Perhaps some additional factors may include those that are associated with fearful behavior (e.g., attention mechanisms), alternate aspects of child emotion regulatory behavior (Buss, 2011), or environmental stressors (Buss et al., 2011).

One factor measured in the current study that was associated with both toddler fear and sadness, as well as cortisol reactivity was caregiver protective behavior. As previous research suggests, it seems when caregivers exhibit higher levels of protective behavior, possibly functioning as overprotection, toddlers display distress expressions and behaviors. Previous research has shown that through biological and environmental factors, mothers' own distress relates to children's distress behavior (e.g., Rubin et al., 2002). A large amount of this research focuses on the maintenance of fear related behavior and anxiety, and our

findings are consistent, suggesting that parents' overprotective behavior, which may reflect parents' own stress, is related to child fear behavior in novel contexts. Perhaps protective behavior is the mechanism for fear and fear-related behavioral outcomes, but not necessarily for fear and stress reactivity. Moreover, our findings further suggest that caregiver protective behavior is also related to child sadness, another type of behavioral distress. Little research has examined these distinct emotions independently in relation to caregiver protective behavior, and our findings suggest that children experience both types of emotion during novel interactions while caregivers display overprotective behavior.

Although child behavioral expressions and protective behavior were examined concurrently in the current study, and therefore causal assumptions about associations cannot be made, Buss and Kiel (2011) used micro-coding to determine the pattern of toddler distress within novelty episodes and provided evidence consistent with the notion that caregiver protective behavior maintained subsequent toddler distress. They suggested that caregivers who displayed lower levels of protective behavior and more encouragement of interaction with novelty may have children who learn to cope and behave more adaptively than children whose mothers exhibited relatively higher levels of protective behavior. Moreover, Kiel & Buss (2011; in press) found that caregiver protective behavior was the mechanism for explaining the association between fear and later anxiety behaviors. Thus, evidence suggests that perhaps caregiver protective behavior occurs prior to children's negative emotion displays.

The current study differs from these previous studies by measuring stress reactivity in addition to behavioral distress in relation to caregiver protective behavior. The association between higher levels of caregiver protective behavior and increased cortisol reactivity in toddlers is consistent with previous research suggesting that continuous stress in the parent-child relationship, unsupportive parent-child interactions, and ineffective caregiving are associated with increased cortisol reactivity, particularly in low-risk environments (e.g., Essex et al., 2002; Repetti, Taylor, & Seeman, 2002). Even in less severe environments, too much protective behavior is thought to restrict and intrude on children's activities (Rubin et al., 2002). Children of parents who consistently display this type of caregiving behavior may not be learning effective coping strategies to manage behavioral and physiological regulation, and instead experience increased cortisol reactivity when their parents model these types of protective behaviors and likely reinforce distress.

Consistent with expectations, caregiver protective behavior accounted for the association between child sadness and cortisol reactivity. Given previous research showing that distress occurred following caregiver overprotective behavior (Buss & Kiel, 2011) and that ineffective caregiver behavior has consistently been linked to cortisol reactivity, it is not surprising that caregiver protective behavior played an important role in explaining the links between child behavioral distress and cortisol reactivity. Research suggests that children's behavioral and physiological distress reactions are related to parents' responses through biological and environmental processes. Perhaps one explanation for findings from the current study may be that some of these toddlers are exposed to chronic levels of overprotection and become sensitized to it over time, particularly in stressful and distressing contexts. It has been suggested that when children are with parents who are sensitive and attentive, they are able to communicate distress in ways which are adaptive, without resulting in physiological stress responses such as cortisol reactivity (Gunnar & Donzella, 2002). Examining caregiver protective behavior and toddler distress with temporal precision may allow for further understanding of the mechanisms underlying the current findings.

While it is clear that toddler emotion has important implications for behavioral distress and cortisol reactivity, results from the current study would suggest that it is actually caregivers'

responses during these moments of distress that are related to cortisol reactivity. It has been asserted that cortisol is not necessarily an emotional physiological response, but rather a physiological stress reaction that responds to different types of emotions and threat depending on developmental period and context (Gunnar & Adam, 2012). Therefore, it is important to consider contextual factors (such as social support and caregiver responses) that may be affecting cortisol, instead of simply examining emotional displays.). In addition, it is possible that cortisol reactivity varies with the intensity of an emotion response, as opposed to the distinct emotion. Sadness, which includes crying, may be thought of as a high intensity response whereas fear is a lower intensity response. The current research indicates that further distinguishing long-term behavioral and physiological profiles of sadness compared with, fearful toddlers is an important direction for future research.

This study had limitations that should be considered. Due to difficulty collecting cortisol from toddlers (i.e., refusals, not enough sample to analyze), the sample size is relatively small. A larger sample may allow for a more powerful analysis that detects stronger effects. Moreover, repeated samples of cortisol taken more often throughout the laboratory visit would offer more evidence for temporal relations, and would discount any possible carryover effects from the different contexts or specific effects from the contexts 15–20 minutes prior to the post-sample. Examining these associations longitudinally would allow for causal conclusions to be drawn about the direction of effects of caregiver protective behavior on behavioral distress and cortisol reactivity. Caregiver protective behavior was also a behavioral variable, and did not account for mothers' cognitions. This type of information could reveal the motivation for mothers' overprotective behavior (restriction of autonomy vs. extreme parental focus on child emotion). In addition, the community sample presumably includes parents with low levels of anxiety, which likely limited the frequency and intensity of protective behavior displayed during different episodes. Using a clinical sample of parents with higher levels of anxiety may allow for more powerful effects of caregiver protective behavior on the associations between child distress and cortisol reactivity. In addition, this sample was predominately of European American descent and middle class, thus findings cannot be generalized to a more diverse sample. Future studies should expand samples to more ethnically and socioeconomically diverse populations. Additional contextual factors should be investigated that may also play a role in accounting for associations between child distress and cortisol reactivity. Work examining other physiological systems that respond to stress may also be useful in understanding the significance of early caregiver behavior on the development of physiological regulatory systems in children.

In conclusion, the current study showed that caregiver protective behavior, which functions to prevent a child from interacting with a novel stimulus, is an important mechanism in explaining the association between toddler sadness and toddler cortisol reactivity during novel contexts. Previous research suggests that caregiver protective behavior predicted increased displays of distress for toddlers in novel situations, and the current study extended these findings to suggest that caregiver protective behavior additionally relates to increased levels of cortisol reactivity in children, and explains associations between child sadness and cortisol reactivity. This evidence suggests that perhaps when parents allow children to experience and express distress appropriately, instead of preventing it, children may learn adaptive strategies to cope with distress that do not result in a physiological stress response.

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Figure 1.

Scatterplot of the correlation between child sadness and cortisol reactivity.

* $p < .05$; ** $p < .01$; *** $p < .001$

Figure 2.

Standardized (unstandardized) regression coefficients for the Sobel test for mediation of protective behavior in the association between toddler sadness and cortisol reactivity.

Table 1

Means, standard deviations, minimum values, and maximum values of variables of interest

Variable	Mean	Std Dev	Min	Max
Toddler Sadness	7.23	8.46	.00	41.79
Toddler Fear	27.92	12.08	1.29	60.29
Caregiver Protective Behavior	.07	.13	.00	.99
Pre-Cortisol Sample	.17	.11	.04	.61
Post-Cortisol Sample	.14	.09	.03	.55
Toddler Cortisol Reactivity	.14	.90	-.88	3.74

Table 2

Correlations among toddler sadness and fear, caregiver protective behavior, and cortisol reactivity.

	Sadness	Fear	Protect	Cort
Toddler Sadness	---			
Toddler Fear	.25*	---		
Caregiver Protective	.54**	.24*	---	
Cortisol	.40**	.24	.39**	---

*
p < .05,**
p < .01