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Individual Differences in Preschoolers' Salivary Cortisol and Alpha-Amylase Reactivity: Relations to Temperament and Maladjustment

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Abstract

We examined the relations of 84 preschoolers' (43 boys; mean age = 54 months) situational stress reactivity to their observed emotions and mothers' reports of temperament and adjustment. Salivary cortisol and salivary alpha-amylase (sAA) were collected prior to, and following, a frustrating task. Children's anger, sadness, and positive affect were measured, and mothers reported on preschoolers' dispositional emotionality, regulation, impulsivity, and problem behaviors. Forty-seven percent of children had an increase in sAA and 52% had an increase in cortisol following the challenging task. On average, sAA levels showed the predicted pattern of rise following the frustrating task, followed by return to baseline. For cortisol, there was a mean increase from pre-task to 40 minutes post-test. sAA reactivity was associated with relatively low levels of dispositional anger and impulsivity and relatively high regulation, particularly for girls. sAA reactivity also was related to low externalizing problems for girls, but not boys. Although cortisol reactivity was unrelated to children's emotions and maladjustment, it was positively related to mothers' reports of regulation. The findings suggest that sAA reactivity in response to a frustrating social task may reflect girls' constrained behavior.

In recent years, a growing body of literature has advanced our understanding of the role of emotional reactivity and regulation on children's social functioning (Eisenberg, Spinrad, et al., 2004; Rothbart & Bates, 2006; Spinrad et al., 2007). Increasingly, investigators have employed multi-level measurements of behavioral *and* psychobiological reactivity and regulation in an effort to further advance theory (e.g., Cicchetti & Blender, 2004). Thus far, the majority of empirical work has focused on integrating minimally invasive measures (in saliva) of the activity of the hypothalamic-pituitary-adrenocortical (HPA) axis; however, technical advances have recently enabled developmental researchers to also include salivary measures of the activity of the sympathetic nervous system (e.g., Granger et al., 2007). The present study is among the first to examine the correlates and concomitants of salivary markers of *both* the HPA axis and the SNS in preschoolers in response to a social frustration.

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One of the main components of the psychobiology of stress involves the activation of the HPA axis and release of cortisol from the adrenal glands into the bloodstream (e.g., Kirschbaum, Read, & Hellhammer, 1992). Recent meta-analyses reveal that, on average, cortisol levels rise in response to social evaluative threat (e.g., Dickerson & Kemeny, 2004). Yet, individual differences are the norm (Granger et al., 1994; Gunnar et al., 2003; van Goozen et al., 2007), and consequentially, a variety of person variables have been suggested to moderate HPA activation, such as temperament, psychopathology, previous experiences, and perceived control. For instance, higher levels of HPA activation have been linked to higher ratings of internalizing problems, withdrawn temperament (shyness), and internalizing emotions (Davis, Donzella, Krueger, & Gunnar, 1999; Granger et al., 1996; 1998; Kagan et al., 1987; 1988; Smider et al., 2002). In clinical, high-risk, and normative samples, lower levels of HPA activation have been associated with higher levels of externalizing problem behavior (Hart, Gunnar, & Cicchetti, 1995; Shirtcliff et al., 2005; Van Goozen et al., 1998; 2000) suggesting that children who are prone to aggression may be “under-aroused” (Raine et al., 1990). Under conditions of novelty, however, cortisol elevations have been positively associated with externalizing behaviors (de Haan, Gunnar, Tout, Hart, & Stansbury, 1998). Researchers also have found that cortisol is associated with relatively low effortful control (Donzella, Gunnar, Krueger, & Alwin, 2000; Gunnar et al., 1997) and high impulsivity/surgency (Gunnar et al., 2003).

The second major component of the psychobiology of the stress response involves activation of the sympathetic branch of the autonomic nervous system (SNS). Recently, there has been renewed interest in salivary alpha-amylase (sAA) as a minimally invasive surrogate marker of SNS activation (see Granger et al., 2007 for review). To more adequately examine the psychobiological markers of stress reactivity, researchers have argued that it is important to utilize multiple measures of the stress-related biological processes (e.g., Bauer et al., 2002). Indeed, several studies reveal that individual differences in sAA levels or reactivity moderate associations between cortisol and children's behavior (El-Sheikh et al., 2008; Gordis et al., 2006). Because the HPA and SNS show different patterns of response to threat and challenge, rates of activation and recovery are faster for sAA than for cortisol, with sAA reaching a peak at around 10 minutes post-stress (or sooner) and about 20 minutes post-stress for cortisol (Gordis et al., 2006; Granger et al., 2007). Thus, among the objectives of the current work was to employ a multi-measure approach and test the relation of *both* sAA and cortisol reactivity to socioemotional functioning.

The study of sAA as a measure of stress reactivity is a relatively new phenomenon in developmental science; only a few researchers have examined the relations of sAA to social functioning in young children. In two recent investigations, sAA and cortisol interacted to predict problem behaviors (El-Sheikh, Buckhalt, Erath, Granger, & Mize, 2008; Gordis et al., 2006). It is important to continue to study the relations of sAA to children's problem behaviors because both aforementioned studies included older children (school-aged or adolescents), and no studies to date have included measures of sAA reactivity and both internalizing and externalizing problems. Moreover, there has been no work to date on the relations of sAA reactivity to situational and dispositional emotions and regulation in a preschool-aged sample (Davis & Granger, in press; Fortunato, Dribin, Granger & Buss, 2008).

Given the lack of research on sAA in young children, strong conclusions cannot yet be drawn regarding the relations of sAA reactivity to children's emotion, regulation, impulsivity and problem behaviors. Thus, findings on other SNS markers are useful in making predictions. For example, skin conductance and preejection period (PEP) have been used as a proxy for SNS (e.g., Berntson, Cicioppo, & Quigley, 1994). Researchers have found baseline or reactive skin conductance to be associated with relatively high levels of internalizing problems (Kagan et al., 1987) and fearfulness (Fowles, Kochanska, & Murray, 2000). In addition, PEP has been

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associated with higher freezing behavior (an indicator of fearful temperament; Buss, Davidson, Kalin, & Goldsmith, 2004) as well as relatively low levels of externalizing problems (Boyce et al., 2001). These studies suggest that SNS reactivity may be related to high internalizing and low externalizing problems.

Researchers often have found gender differences in children's negative emotions and problem behaviors, especially the latter (Crijnen, Achenbach, & Verhulst, 1997; Else-Quest, Hyde, Goldsmith, & Van Hulle, 2006; Stanger, Achenbach, & Verhulst, 1997). One possible mechanism for these gender differences may be that boys and girls respond to stress differently (Stroud et al., 2002). Specifically, Booth et al. (2008) posit that females respond to challenge by focusing more on managing interpersonal relationships (building alliances, calming others) whereas males, when faced with stress, tend to engage in risky behaviors (see Taylor et al., 2000). Thus, boys' and girls' stress responses may have different implications for their social functioning. As a case in point, Smider et al. (2002) found that baseline cortisol levels predicted higher withdrawal behavior for girls, but not boys. On the other hand, trait-level cortisol has been found to predict lower externalizing problems for boys, but not girls (Shirtcliff et al., 2005) and higher negative affect in girls, but not boys (Quas, Hong, Alkon, & Boyce, 2000). Moreover, Vigil and colleagues (in press) found that sAA was negatively related to depression in male adolescents but was positively related to depression in female adolescents, suggesting that girls who are physiologically reactive may be at heightened risk for internalizing problems.

The purpose of the present study was to investigate preschool children's cortisol and sAA reactivity following a frustrating task and to examine the relations between cortisol and sAA reactivity to children's observed emotions and maternal reports of dispositional emotionality, regulation, impulsivity, and maladjustment. We expected cortisol and perhaps sAA to be related to higher levels of internalizing problems and negative affect (especially internalizing emotions rather than anger) and lower levels of effortful control, impulsivity, and externalizing problems. In addition, we were interested in analyzing whether the relations between stress reactivity and children's emotions and social functioning differed for boys and girls.

Finally, Bauer et al. (2002) suggested that a multi-system approach to studying biological processes is needed, and that interactions between HPA and SNS activity may predict individual differences in social behavior. Thus, as a final goal, we tested whether preschool children's cortisol moderated the relations between sAA and children's emotions, regulation, and adjustment.

Method

Participants

Participants were preschool-aged children residing in a large Southwestern city who were part of a longitudinal study of children's social and emotional development. Children were approximately 4.5 years of age at the laboratory visit (mean = 54.07 months, *SD* = .97). Initially, families were recruited through three local hospitals following the birth of the target child. The current study consisted of 84 children who completed saliva collection at the preschool assessment (43 boys, 41 girls). The majority of the children in the current study were White, non-Hispanic (76.2%), and the remaining children were identified as Hispanic (10.7%), African American (3.6%), Native American (2.4%) or other/mix of two races (9.5%). Family income ranged from less than $15,000 to over $100,000 (median income ranged from $45,000 to $60,000). The majority of parents were married (78%), and those parents were married an average of 8.87 years (*SD* = 4.70). Mothers' average age was 33.74 (*SD* = 5.06) and fathers' age was 42.20 (*SD* = 5.16) at the laboratory assessment.

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Because we began including saliva data midway through data collection, the participants in this study included only 50% of the children who participated in the preschool laboratory visit (n = 168). We did not expect any differences in the children who had saliva data (n = 84) versus those who did not (n= 84). As expected, *t*-tests revealed no significant differences in demographic or study variables. We also examined whether there were differences in children who provided saliva samples from those who were enrolled in the study at the initial laboratory visit (i.e., at 18 months of age) but did not provide saliva samples. *T*-tests were conducted and revealed no differences between the groups on demographic or study variables.

Procedure

Children and their primary caregiver came to a research laboratory room. The parent was escorted to a separate room to complete questionnaires regarding children's temperament and social competence and adjustment. A trained female undergraduate experimenter conducted all of the laboratory tasks. Children participated in a series of tasks designed to assess children's effortful control, social competence, and cognitive developmental level (not used in the current analyses). Immediately before the first saliva collection, children participated in a series of executive functioning tasks that required them to inhibit a predominant response behavior. Specifically, the children were trained to imitate the experimenter either knocking or tapping (flat hand) on the table. After the training, the rules to the game changed so that the child was told to knock when the experimenter tapped and to tap when the experimenter knocked on the table. In the next executive functioning task, the child was instructed to say the word, “grass” when the experimenter held up a white card and to say, “snow” when the experimenter held up a green card. These tasks were presented as games and were not expected to elicit negative emotion.

After the executive functioning tasks, the experimenters then collected a saliva sample (see below). Next, the children participated in an emotion-eliciting task from the Preschool Laboratory Assessment Battery (Buss & Goldsmith, 2000; Lemery et al., 1999) and provided a second saliva sample approximately 10 minutes following the stressful task. A third saliva sample was collected at the end of the laboratory visit, approximately 40 minutes following the task. At the end of the session, children received a small gift, and parents were paid for their participation.

Measures

Emotion-eliciting task—Children participated in the “not-sharing” task from the preschool version of the Laboratory Temperament Assessment Battery (Goldsmith, Reilly, Lemery, Longley, & Prescott, 2003). At the beginning of this paradigm, the experimenter was given treats (i.e., candy) to be shared *equally* between the experimenter and the child. The experimenter was seated across from the child and held the container of candy and two clear jars. The experimenter designated a jar for herself and one for the child, and proceeded to divide the candy between the jars. Initially, the candy was divided equally, but then the experimenter began to divide the pieces unfairly, giving more candy to herself. During this unfair portion, the experimenter took one of the child's pieces of candy and ate it. The task culminated in the experimenter taking all of the candy (including what was already distributed to the child). At the conclusion of the assessment, the experimenter explained that she was being unfair and offered the child three pieces of candy to take home.

Saliva collection—As noted above, saliva samples were collected prior to the not-sharing task (pre-test) and at approximately 10- and 40-minutes following the task. Samples were collected using a small micro-sponge device that had a short plastic applicator shaft (0.4 × 5.2 mm) that served as a handle (Granger et al., 2007). Attached to the end of the handle was a small (0.7 × 1.8 mm) arrowhead shaped sponge that was placed under the child's tongue. In

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order to keep the children's attention and to increase children's compliance with the saliva collection procedure, the experimenter prompted the child to “guess the flavor” of the stick while mouthing two micro-sponges simultaneously for 90 seconds. The experimenter demonstrated how to hold the stick in place using a coffee-stirring straw and continued to encourage the child throughout the 90-seconds. At the end of each collection period, the child was asked to guess the flavor and was given a small prize (a sticker after the first two collections and a lollipop at the final collection). Samples were placed in an air-tight storage container and frozen at a minimum of -20 degrees centigrade. Samples were shipped to the Behavioral Endocrinology Laboratory at Pennsylvania State University where they were stored at -80 degrees centigrade in locked freezers until they were assayed.

Salivary alpha-amylase—Samples were assayed for alpha-amylase using a commercially available kinetic reaction assay (Salimetrics, State College, PA). The assay employed a chromagenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. The enzymatic action of alpha-amylase on this substrate yielded 2-chloro-p-nitrophenol, which was spectrophotometrically measured at 405 nm using a standard laboratory plate reader. The amount of α-amylase activity present in the sample is directly proportional to the increase (over a 2 minute period) in absorbance at 405 nm. The test required 10 μL of sample, and 3 children did not provide sufficient sample volume of saliva to be assayed. In addition, 1 child had missing data at the 10-minute follow-up, and 3 outliers on the level of sAA (defined as those scores that were at least 3 standard deviations away from the mean) were removed from analyses, resulting in 77 children with sAA data (one additional child was missing alpha-amylase data from the 40 minute follow up; however, this variable was not used in further analyses, see below).

The time course of the salivary alpha-amylase response to stress is much faster than cortisol (peaking at approximately 10 minutes following the stressor or sooner; Stroud et al., 2009); thus, we computed a salivary alpha-amylase reactivity score by computing the difference between sAA levels prior to the not-sharing task (pre-test) and sAA levels at the 10-minute post test.

Cortisol—All samples were assayed for salivary cortisol by enzyme immunoassay (Salimetrics, State College, PA). This test required 25 μl of saliva that has a range of sensitivity from .007 to 3.0 μg/dL, and average intra-and inter-assay coefficients of variation 5% and 10% respectively. Six children did not provide sufficient sample volume of saliva to be assayed for salivary cortisol, and 3 outliers (on level of cortisol) were removed from analyses, resulting in 75 children with cortisol data (one additional child was missing cortisol data and another child did not provide sufficient sample volume from the 10 minute-follow-up; however, this variable was not used in further analyses; see below).

To calculate the cortisol reactivity score, we computed a difference score between cortisol levels at the pre-test and cortisol levels at the end of the laboratory visit (approximately 40 minutes post-test), given the relatively slow rate of activation and recovery of salivary cortisol to stress (Granger et al., 2007; Gordis et al., 2006; Stroud et al., 2009).

Children's observed emotion—Children were observed during the “not-sharing” task, taken from the preschool version of the Laboratory Temperament Assessment Battery (Goldsmith, Reilly, Lemery, Longley, & Prescott, 1999). Coders rated children's anger, positive affect, and sadness using the “unfair” portion of the not-sharing task. Emotions were coded on a 4-point scale (1= *no emotion*; 4 = *intense emotion*) in 10-second epochs, and these scores were subsequently averaged across the episode. Children's *anger* was scored as 1 if the child did not exhibit anger, 2 if the child exhibited low intensity or very brief anger (facial expression or verbalization), 3 if the child exhibited moderate anger (moderate intensity facial

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expression or verbalization or bodily movement such as stomping fist), and 4 if the child expressed intense or prolonged anger. Children's *sadness* was scored as 1 if the child did not exhibit any sadness, a 2 reflected low intensity or brief sadness, either facially, verbally, or bodily (slump or dropping head), a 3 represented moderate or more prolonged sadness, and a 4 indicated intense sadness. *Positive* emotion was coded as a 1 if no positive emotion was observed, 2 was coded to reflect low intensity positive emotion, such as a brief smile or positive vocalization, 3 indicated moderate intensity positive affect or more prolonged positive emotion, and 4 reflected intense positive emotion, such as an intense smile, laughter, or prolonged smiles or vocalizations (squealing). Inter-rater reliability was calculated on 27% of the videotapes, and intraclass correlations for anger, sad, and positive emotion were .87, .90 and .94, respectively. The not-sharing task clearly elicited negative emotion in the children. We found differences in children's positive emotion, anger and sadness across the fair and unfair portions of the task, *F*s ranged from 26.66 to 55.85, *p*s < .001 (*M*s for anger, sadness, and positive affect in the fair segment = 1.01, 1.00, 1.46; *M*s for unfair section = 1.13, 1.23, and 1.30, respectively).

Children's dispositional regulation, impulsivity, and emotion—Mothers rated (1 = *never* to 7 = *always*) items from three subscales of the Child Behavior Checklist (CBQ, Rothbart et al., 2001). The subscales were attention focusing (14 items, e.g., "When drawing or coloring in a book, shows strong concentration"), attention shifting (12 items, e.g., "can easily shift from one activity to another"), and inhibitory control (13 items, e.g., "is good at following instructions"). Alphas were .77, .73, and .80 for the attention focusing, attention shifting, and inhibitory control subscales, respectively. Mothers' reports of inhibitory control were significantly positively correlated with mothers' reports of attentional shifting and focusing, *r*s(80) = .50, and .61, respectively. The subscales were standardized and averaged to form an effortful control composite. In order to measure children's reactive undercontrol, mothers also completed the impulsivity scale (13 items, e.g., "often rushes into new situations", alpha = .75).

In addition, mothers' rated children's dispositional emotions of positive emotion, anger, and sadness from the CBQ. The smiling and laughter subscale consisted of 13 items representing children's positive emotion, (e.g., "laughs a lot at jokes and silly happenings," alpha = .79). The anger/frustration scale was composed of 13 items (e.g., "has temper tantrums when s/he doesn't get what s/he wants", alpha = .80), and the sadness scale had 13 items (e.g., "cries sadly when a favorite toy gets lost or broken," alpha = .74).

Children's maladjustment—Mothers completed the Infant/Toddler Social and Emotional Assessment (ITSEA; Carter et al., 2003) to assess children's maladjustment. Items are rated on a 3-point scale (0 = *not true*; 2 = *very true*). The externalizing scale consisted of three subscales including activity/impulsivity (6 items), aggression/defiance (12 items), and peer aggression (6 items). The internalizing scale consisted of 4 subscales including separation distress (6 items), inhibition to novelty (5 items), depression/withdrawal (9 items) and general anxiety (12 items). Alphas for the scales were .87 and .80 for externalizing and internalizing, respectively.

Results

Descriptive Data

The means and standard deviations for the study variables are presented in Table 1. To determine whether there were changes in children's salivary cortisol and sAA levels across the three saliva collection points, repeated measures ANOVAs were conducted. For salivary cortisol, there was a significant within-subjects main effect for saliva sample timing, $F(2, 144) = 9.65$, $p < .01$, indicating that, on average, cortisol levels increased at the 40-minute post-test,

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relative to the pre-test and 10-minute post-test; both the linear and quadratic effects were significant, $Fs(1, 72) = 9.36$ and 10.49, $ps < .01$. Post-hoc pairwise comparisons using LSD difference revealed significant differences between the 40-minute post-test and the other saliva collection points, $ps < .01$.

For sAA, there was also a main-effect for saliva sample, $F(2, 150) = 5.51$, $p < .01$, indicating the expected increase in sAA between the pre-test and 10-minute post-test and return to baseline levels at the 40-minute post-test; the quadratic effect was significant, $F(1, 75) = 7.07$, $p < .01$. Posthoc pairwise comparisons using the LSD difference statistic indicated that there was a significant difference between the 10-minute post-test and the 40-minute post-test, $p < .01$, and a marginally significant difference between pre-test and 40-minute post-test, $p < .06$. The difference between the pre-test and the 10-minute post-test was not significant.

An individual differences approach revealed that 52.0% of children's cortisol levels increased between the pre-test and 40-minute post-test by at least 10%, and 32.0% of the children's cortisol levels decreased by at least 10%. Similarly, 46.8% of the children increased in sAA levels and 32.5.0 % decreased by at least 10% from the pretest to the 10-minute post-test.

Because of potential gender differences in psychobiological measures (Stroud et al., 2002) and in children's emotions and maladjustment/adjustment, we examined whether there were differences in the study variables as a function of children's sex. There were no differences in either pre-test levels of cortisol and sAA or in cortisol or sAA reactivity. However, boys and girls differed in their expressed emotion to the not-sharing task. Specifically, boys displayed more anger during the task ($M = 1.20$, $SD = .26$) than did girls ($M = 1.06$, $SD = .13$), $t(79) = 2.99$, $p < .01$. In addition, mothers reported more internalizing problems for their daughters ($M = 1.50$, $SD = .23$) than sons ($M = 1.40$, $SD = .19$), $t(77) = -2.13$, $p < .05$. Moreover, the variances differed for boys and girls in the amount of anger expressed during the not-sharing task, Levene's test = 26.25, $p < .01$, indicating that boys had larger variation in this behavior than did girls. Boys, compared to girls, also had higher variance for externalizing problems, Levene's test = 4.11, $p < .05$.

Relations of Cortisol and sAA to Children's Observed Emotions

To examine whether cortisol and sAA reactivity were associated with children's observed emotions during the not-sharing task, partial correlations (controlling for pre-task levels of sAA or cortisol) were computed. Neither children's cortisol reactivity nor sAA reactivity was related to children's observed emotionality during the not-sharing task. There was a near-significant negative relation between sAA reactivity and girls', but not boys', positive emotion, $p = .08$ (see Table 2).

Relations between Cortisol and sAA to Children's Dispositonal Emotionality, Effortful Control and Maladjustment

We also examined the relations between sAA and cortisol reactivity with maternal reports of dispositional emotion, effortful control, and impulsivity or adjustment, controlling for initial levels of cortisol or sAA (see Table 3). Both cortisol and sAA reactivity were positively related to children's effortful control. In addition, there was a significant negative relation between sAA reactivity and children's dispositional anger. When examining the relations separately for boys and girls, the relations between sAA reactivity and children's dispositional anger and effortful control were significant for girls, but not boys, and the comparison between the correlations for boys and girls was significant. Similarly, there was a negative relation between sAA reactivity and impulsivity for girls, but not boys (the comparison between the correlations was marginally significant).

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No relations were found between cortisol reactivity and mothers' reports of maladjustment. However, sAA reactivity was negatively related to girls', but not boys', externalizing problems, and the comparison between the correlations for boys and girls was marginally significant (see Table 3). Internalizing problems were not related to cortisol or sAA reactivity.

The Moderating Relation of sAA and Cortisol to Children's Emotion and Adjustment

Regressions were computed including the main effects of sAA reactivity and cortisol reactivity and their interaction on children's emotions and adjustment. The interactions were not significant; in addition, the three-way interaction between sAA, cortisol and children's sex was not significant.

Discussion

The purpose of the present study was to examine preschoolers' stress reactivity using two biological markers, cortisol and sAA. The results of this study add to our knowledge of the correlates of biobehavioral markers of stress in young children. Specifically, the findings indicate that sAA is a useful measure of reactivity in preschool-aged children and is related to children's higher dispositional regulation and lower externalizing problems. Moreover, the findings of this study highlight the need to examine children's sex when investigating the relations of stress reactivity to children's outcomes.

The results of this study support the expected pattern of stress reactivity for both cortisol and sAA, with an average increase in both sAA and cortisol following the not-sharing task. Moreover, the findings of this study also showed that individual differences in sAA reactivity were associated with girls' *lower* dispositional anger and *higher* effortful control. These findings indicate that sAA reactivity may reflect young children's constrained or inhibited behavior. Indeed, Aksan and Kochanska (2004) argued that behavioral inhibition predicts low impulsivity, which in turn, contributes to the development of effortful control. It is also possible that if sAA reactivity reflects higher behavioral inhibition, then adults may simply view these children as more regulated and controlled. Another possible explanation for these findings is that sAA reactivity is appropriate in response to the social frustration task, and thus, is related to children's relatively high regulation skills.

In addition, sAA reactivity was negatively related to girls' (but not boys') externalizing problems as reported by mothers. The negative relation between biological markers of stress reactivity and externalizing problems also has been shown in the existing literature (Gerra et al., 1998; Granger et al., 1996; Moss et al., 1995; van Goozen et al., 2000), suggesting that that children who have high levels of arousal may be protected against the development of antisocial behavior. In other words, children who have a low threshold for physiological arousal may withdraw from the social interaction, but that children who have higher thresholds for reactivity behave aggressively as a way to seek sensation (Granger et al., 1998; Raine, 2002).

The fact that the negative relation between sAA reactivity and externalizing problems was only significant for girls raises another interesting issue. Gender appears to play an important role in understanding the relations between reactivity and children's outcomes. Although the sex differences found in this study were relatively weak, the findings from this and other studies show that boys and girls deal with stressful or challenging situations in different ways (Booth et al., 2008; Murray-Close & Crick, 2007; Shirtcliff et al., 2005; Vigil et al., in press). That is, girls may deal with challenge by withdrawing from social interactions whereas boys may behave aggressively (see Booth et al., 2008). Thus, researchers clearly need to have gender-specific models for explaining predictors of children's problem behaviors. Because externalizing problems generally are less common and less consistent with gender roles for girls than boys (Moffit, Caspi, Rutter, & Silva, 2001; although the difference was not significant

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in this study), high levels of externalizing problems may be more problematic for girls. Thus, it is possible that stress reactivity is an important mechanism to examine when understanding risky behavior in girls.

Perhaps more unexpected was our finding that cortisol reactivity was positively linked with effortful control. Prior work has shown cortisol (baseline and changes throughout the day) to be linked with lower effortful control (Dettling et al., 1999; Donzella et al., 2000; Gunnar et al., 1997; 2003). Perhaps cortisol arousal during this unfair task represents children's allocation of attentional resources, internal cognitive processes, and active engagement in the task. Thus, rather than viewing cortisol elevations as a failure to cope with the stressful task, it is possible that cortisol responding to this social task may reflect children's assertion or dealing with their unfair treatment. Thus, it is possible that the small increases in cortisol reactivity in response to this task are adaptive, as opposed to larger or more chronic increases that may be evident in more fear-inducing or higher-stress situations.

It is interesting to note that cortisol reactivity was unrelated to children's maladjustment. There are at least a few reasons why this might be the case. In prior work, HPA activation has been consistently related to measures of withdrawal or internalizing emotions (Buss & Goldsmith, 2007). Because the stressful task was designed to elicit frustration and anger (as opposed to inhibition and withdrawal), the nature of the task may have prevented associations with cortisol (see Lewis & Ramsay, 2005; Lewis et al., 2006). In contrast, it has been suggested that sAA reactivity may be correlated with the most intense emotion appropriate for the task. This explanation is supported by recent work of Fortunato et al. (2008), who found sAA to be positively related to positive affect during a series of tasks in which positive affect and approach were the predominant behaviors observed in the tasks. Thus, sAA may be situation-specific and depend on contextual factors. In the present study, anger and low positive emotion would be expected in response to the “not-sharing” task, and sAA was nearly significantly related to low positive emotion during this task (for girls).

Another reason for the lack of findings for cortisol may be due to the timing of our data sampling. Because our final sampling of saliva was at approximately 40 minutes post-test, we may have missed the peak level of cortisol following the stress (which typically occurs approximately 20 minutes after the stress; Gordis et al., 2006; Stroud et al., 2009). We chose to collect the final saliva sample at the end of the visit because we felt that we would have more compliance with the saliva collection after the children had completed more non-stressful and fun tasks. Although cortisol significantly increased between the pre-test and the 40-minute post-test, it is possible that cortisol was already beginning to recover by this sampling point. In the future, researchers could collect multiple saliva samples following the challenging task to more accurately measure children's peak cortisol reactivity.

It is also important to note that our data sampling procedure may have diminished findings for both sAA and cortisol. The tasks that preceded the not-sharing task (i.e., executive functioning tasks) may have elicited interest and attention in the children. Thus, it is certainly possible that our first saliva sample was not a clear “baseline,” as children may have reacted with an increase in sAA (or perhaps cortisol) following the executive functioning tasks. Because sAA has been viewed as a more sensitive measure of reactivity than cortisol (Mize, Lisonbee, & Granger, 2005), this argument may be especially applicable for sAA. Thus, future research should examine preschoolers' reactivity to stress by utilizing clear baseline measures.

We also did not find that sAA and cortisol interacted in the prediction of children's maladjustment, as has been suggested by Bauer and colleagues (Bauer et al., 2002). One reason for the lack of findings may be that our small sample size limited the statistical power to detect such interactive effects. Moreover, it is possible that our relatively mild stressor did not elicit

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enough HPA reactivity to find such moderating effects. Future work in this area should examine both sAA and cortisol reactivity in response to tasks that are designed to elicit high levels of stress, such as introducing a frightening person or witnessing adults arguing.

Although the current study provides an important step to understanding the role of sAA reactivity in preschoolers' socioemotional functioning, our study is limited in several ways. First, our study was limited by a small sample size and missing data, reducing the statistical power, particularly when examining boys' and girls' separately. Our research should be replicated with a larger sample. Second, the nature of this study did not allow us to examine *changes* in children's reactivity with age or to determine if children's stress responses predict children's outcomes later in development. Thus, longitudinal studies that include biophysiological markers of stress over time are needed.

It is also important to recognize that there are likely bidirectional relations between biological markers of reactivity and children's emotions and social functioning. It is possible that children who tend to engage in externalizing problems may become desensitized to social frustrations and thus display low levels of physiological reactivity in response to the task. On the other hand, it may be that low reactivity is responsible for children's underarousal, which in turn, leads to increased risk for aggression and disruptive behavior (Raine, 2002). Thus, it is important to acknowledge potential bidirectional effects. Further longitudinal work that controls for earlier levels of stress reactivity and problem behaviors when predicting outcomes such as maladjustment across time may help to disentangle some of these issues.

Despite these limitations, this study contributes to our understanding of biological markers of reactivity in preschool-aged children. This study points to the usefulness of measuring sAA and cortisol as tools to examine children's reactivity to challenging situations.

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Table 1

Means and Standard Deviations of Study Variables

Variable	Mean	SD	Min	Max	N
Cortisol (Ug/dL)					
Pre-test	.08	.04	.00	.24	75
10-minute post-test	.08	.04	.00	.17	73
40-minute post-test	.09	.05	.00	.25	75
Salivary Alpha-amylase (U/mL)					
Pre-test	38.07	30.69	1.50	139.70	77
10-minute post-test	41.87	29.54	3.00	111.80	77
40-minute post-test	33.60	22.44	2.30	99.40	76
Observed Emotion					
Anger	1.13	.22	1.00	1.90	84
Sadness	1.23	.28	1.00	2.33	84
Positive	1.30	.27	1.00	2.00	84
Maternal Reports					
Dispositional anger	4.64	.78	2.54	6.62	82
Dispositional sadness	3.98	.78	1.69	6.00	82
Dispositional positive	5.78	.53	4.31	6.62	81
Effortful control	4.51	.56	3.27	5.78	82
Impulsivity	4.71	.67	3.23	6.15	82
Externalizing	1.54	.27	1.00	2.47	82
Internalizing	1.46	.21	1.00	2.14	82

Note. Two mothers did not complete the questionnaire packet (and one mother was missing items from the smiling/laughter scale).

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Table 2

Partial Correlations Between Salivary Alpha-Amylase And Cortisol Reactivity To Children's Observed Emotion During The Not-Sharing Task For Boys And Girls, Controlling For Initial Task Levels Of Cortisol Or sAA

	Cortisol Reactivity			**sAA Re activity**		
	Total	Boys	Girls	Total	Boys	Girls
Observed anger	.15	.19	.10	-.03	-.12	.15
Observed sadness	.07	.26	-.05	-.02	-.08	.05
Observed positive	.00	-.07	.09	-.11	.01	-.29+

Note. n =75 for cortisol analyses (38 boys, 37 girls) and n = 77 for alpha-amylase (38 boys, 39 girls); + $p < .10$.

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Table 3

Partial Correlations Between Salivary Alpha-Amylase And Cortisol Reactivity To Children's Dispositional Emotion, Regulation, Impulsivity And Mothers' Reports Of Maladjustment, Controlling For Initial Task Levels Of Cortisol Or sAA

	Cortisol Reactivity			**sAA Reactivity**		
	Total	Boys	Girls	Total	Boys	Girls
Dispositional anger	.04	-.04	.14	-.25*	-.01 a	-.47** a
Dispositional sadness	.00	.05	-.03	-.19	-.17	-.21
Dispositional positive	.19	.18	.22	.06	.26	-.15
Effortful control	.25*	.27	.24	.25*	.15	.39*
Impulsivity	.03	-.21	.22	-.12	.07 b	-.33* b
Externalizing problems	.08	-.05	.23	-.18	.00 b	-.43** b
Internalizing problems	-.12	-.12	-.07	-.10	-.24	.02

Note. n =73 for cortisol analyses (37 boys, 36 girls) and n = 75 for alpha-amylase (38 boys, 37 girls);

* $p < .05$,

** $p < .01$;

[a] correlations for boys and girls significantly different using z′ transformation;

[b] correlations for boys and girls marginally different using z′ transformation