Published in final edited form as:

Biol Psychol. 2011 September ; 88(1): 57–64. doi:10.1016/j.biopsycho.2011.06.008.

Direct and moderating links of salivary alpha-amylase and cortisol stress-reactivity to youth behavioral and emotional adjustment

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Measurement across multiple stress systems has been touted as the *gold standard* for the study of individual differences in the psychobiology of the stress response (e.g., Frankenhauser, Lunberg & Forsman, 1980; Lovallo, Pincomb, Brackett, & Wilson, 1990). However, implementation of this approach has proved difficult, particularly in children. While measurement of hypothalamic-pituitary-adrenal (HPA) axis activity during childhood can be accomplished non-invasively via the collection of salivary cortisol (e.g., Granger et al., 2007; Schwartz, Granger, Susman, Gunnar, & Laird, 1998), until recently, assessments of the autonomic nervous system (ANS) were cumbersome, requiring costly apparatus, complex data processing techniques (e.g., impedance cardiography, electrocardiograms), or the use of invasive procedures, such as the collection of plasma or spinal fluid. For these reasons, scientists have become keenly interested in salivary alpha-amylase (sAA), an enzyme secreted by the salivary glands in response to autonomic stimulation (e.g., Granger et al., 2006, 2007; Rohleder & Nater, 2009).

Numerous studies suggest sAA as a surrogate marker of the ANS response to stress in adults (e.g., Bosch, Veerman, de Geus, & Proctor, 2011; Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Nater et al., 2005; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004; van Stegeren, Rohleder, Everaerd, & Wolf, 2006), and several recent studies extend this evidence to youth (e.g., Davis & Granger, 2009; Fortunato, Dribin, Buss, & Granger, 2008; Gordis, Granger, Susman, & Trickett, 2006, 2008; Keller & El-Sheikh, 2009; Yim, Granger

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& Quas, 2010). Measuring variation in ANS and HPA components of the stress response in saliva allows the possibility of testing biosocial hypotheses in the context of everyday and ecologically-valid social settings without interrupting the meaningful flow of events and experience (Granger & Kivlighan, 2003).

Until recently, the vast majority of child and adolescent stress response studies employed assessments of adrenocortical reactivity without regard for potential alterations in the ANS system. However, it is assumed that the ANS and HPA systems work in coordination to generate the physiologic changes associated with the stress response, but the exact nature of the coordination (additive or interactive; opposing or complementary) is a subject of debate (Granger et al., 2007a).

An emerging body of research has begun incorporating sAA into biosocial models of stress vulnerability in the context of child adjustment. To date, dysregulation of ANS/SNS activation has been linked to both internalizing (Scarpa, Raine, Venables, & Menick, 1997) and externalizing (Boyce et al., 2001; Zahn & Kruesi, 1993) behavior problems in children, with externalizing behaviors, such as aggression, consistently linked to low baseline ANS markers or hyporeactivity (see Ortiz & Raine, 2004). Similarly, studies of adrenocortical diurnal patterns and reactivity show links between higher basal and reactive cortisol and internalizing problems (Granger et al., 1994, 1996) but lower basal and reactive cortisol and externalizing behaviors (e.g., Flinn & England, 1995; Shirtcliff et al., 2005).

Although patterns of single (ANS or HPA) stress system activity have been linked to child and adolescent behavioral and emotional symptoms, contemporary theorists argue that ANS and HPA stress-related biological processes should be measured in tandem to advance understanding of how biological, social, and behavioral processes interact to determine risk or resilience (Bauer et al., 2002; Donzella et al., 2000). In fact, Bauer et al. (2002) indicate that ANS and HPA activity may be obscured by “emotion-specific” ANS and HPA responses. Moreover, ANS and HPA systems may dissociate from each other, resulting in asymmetrical or discordant patterns of reactivity. In one of the first developmental studies to utilize Bauer et al.'s recommended multiple system measurement approach of stress reactivity including sAA, Gordis and colleagues (2006) reported increases in both sAA and cortisol in response to the Trier Social Stress Test in children aged 10 to 14 years. Similar to findings in adults, this study revealed distinct patterns of reactivity, with sAA showing a faster response curve (relative to cortisol). In addition, sAA reactivity moderated associations between cortisol reactivity and parent-reported aggression, such that low levels of cortisol reactivity were positively associated with parent-reported aggression at low, but not at high, levels of sAA reactivity. In addition to the multi-system approach, Gordis' study points out that patterns of stress activity vary by individual characteristics. Therefore, examination of sAA and HPA patterns among children should include measures of child behavioral and emotional functioning.

Examining the role of child anxiety symptoms is particularly salient in studies of stress activity due to the expectation of heightened physiological reactivity among anxious children (Rappaport & Katkin, 1972; Turner, Beidel, & Larkin, 1986). However, some studies indicate that contrary to the expected findings of hyperreactivity, anxious individuals exhibit a smaller sympathetic response to acute laboratory stress (Fisher, Granger, & Newman, 2010; Hoehn-Saric & Mcleod, 1993), despite having comparable physiological activity at baseline. For example, Fisher et al. (2010) found that higher sAA at baseline was associated with lesser sAA change after exposure to an acute stressor. Likewise, HPA reactivity to stress may be affected by the presence of anxiety symptoms. Overall, studies of sAA in children have yet to explore the links between sAA and non-clinical anxiety,

however, associations between anxiety and sAA and other physiological activity are expected for youth.

Notably, a previous report from our group showed *developmental* influences on sAA, cortisol, and cardiovascular responses to performance and peer rejection stressors (citation removed). Overall, adolescents (ages 13+, late puberty/Tanner stages IV-V) displayed greater increases in cortisol, sAA, and heart rate in response to stressors relative to children (ages 7-12, early to mid puberty/Tanner I-III). Developmental influences on diastolic blood pressure (DBP) and cortisol were most pronounced in response to performance stress, while developmental influences on systolic blood pressure (SBP) and sAA were most apparent in response to peer rejection stress. In this previous study, examination of sex differences in cortisol and sAA reactivity yielded no significant findings.

The Present Study

Using individuals from this same cohort, the current study examined additional correlates and concomitants of sAA reactivity in children and adolescents exposed to acute stress (i.e., performance --modified TSST or interpersonal--peer rejection). This study aims to examine two hypotheses and two exploratory questions. First, since little is known about the links between sAA activity and the activity of other stress systems in youth, we examined associations among sAA and saliva cortisol, blood pressure, and heart rate, with the expectation that sAA would be modestly linked to the cardiovascular measures traditionally viewed as downstream measures of autonomic activity, but not with the adrenocortical response. Second, following the Bauer et al. (2002) conceptual model, we sought to examine individual differences in sAA as a moderator of the association between HPA (cortisol) activity and symptoms of emotional and behavioral problems, including child-reported anxiety and parent-reported internalizing and externalizing behaviors. We hypothesized that in accordance with Gordis et al. (2006), sAA reactivity would moderate cortisol reactivity to predict parental reports of externalizing behaviors, especially aggressive behaviors. However, given the lack of multi-system studies addressing child anxiety and other internalizing symptoms, exploratory analyses examined the interaction between sAA and cortisol reactivity in association with internalizing symptoms and child anxiety. Furthermore, a second exploratory analysis addressed whether controlling for symptoms of anxiety affects the association between sAA and cardiovascular measures.

Methods

Participants

Participants were healthy children/adolescents (27 boys, 29 girls) aged 7 to 16 ($M = 12.0$, $SD = 2.4$) from the initial phase of a larger study (citation removed). Exclusion criteria were based on factors known to influence cortisol and cardiovascular reactivity, including the use of oral contraceptives, thyroid medications, steroids, and psychotropic medications (Hibel et al., 2007), as well as tobacco, drug, or alcohol use. Participants with a history of psychological or behavioral problems or current physical illnesses were also excluded from the study. Fluency in spoken English was required. Racial composition of the sample included: 79% Caucasian, 7% African American, 9% multi-racial, and 5% 'other or don't know.' Seventy-eight percent of mothers and 92% of fathers were employed, with an average family income of \$60,000 to \$80,000. Seventy-eight percent of mothers were married; their education level ranged from some high school to completion of a graduate degree.

Procedures

Protocols and procedures were approved by the hospital's Institutional Review Board and are reported at length in (citation removed). Each participant completed a 2-hour “rest” session, in which they habituated to the laboratory and physiological monitors while completing a battery of questionnaires including measures of anxiety and problem behavior symptoms. Participants then returned to the lab on another day (median = 15.5 days) and completed a 2-hour “stress” session. Stress sessions included a 20-25 minute pre-task baseline period where the youth were asked to read (grade K-2 books) or watch G-rated movies and television shows, followed by three stress tasks lasting 20 minutes collectively, and a one-hour recovery period during which participants completed questionnaires and again watched G-rated movies and television shows. Stress tasks consisted of either performance-oriented tasks (i.e., speech, mental arithmetic, and mirror tracing; Allen & Matthews, 1997; Buske-Kirschbaum, Jobst, Wustmans, & Kirschbaum, 1997) or a peer rejection task (three exclusion challenges with gender/age-matched confederates; Stroud et al., 2000). The use of two distinct session types is consistent with the aims of the larger study and the timing of tasks were matched across session types.

Physiological Assessment—Seven to nine saliva samples were taken over the baseline, stress, and recovery periods. Timing of saliva samples was designed to take into account a 20-minute time to peak reactivity for cortisol and a 10-minute time to peak reactivity for sAA (see below). Blood pressure and heart rate recordings were taken at 2-minute intervals during the baseline and stress induction periods and at 5-minute intervals during the recovery period. Self-reported affect was assessed at baseline, during each stress task, and during the recovery period. All sessions began between 14:00 and 17:00 to control for diurnal variation in cortisol and sAA. Participants were asked to refrain from food and drink (besides water) for two hours prior to the stress session, from exercise for 24 hours prior to the session, and from caffeine beginning the evening before the stress session (Klein et al., 2006).

Measures

Determination of salivary cortisol and alpha-amylase—Whole, unstimulated saliva samples were collected from each participant by passive drool over the course of each stress session (Granger et al., 2007b). Following collection, samples were frozen at -80 degrees Celsius until shipment via overnight delivery on dry ice to Salimetrics Laboratories (State College, PA), where they were assayed. Cortisol was analyzed in duplicate using a commercially available enzyme immunoassay without modification to the manufacturer's protocol (Salimetrics, State College, PA), range of sensitivity from .007-3.0 ug/dL, and intra- and inter-assay coefficients of variation less than 5 and 10% respectively. Salivary cortisol data are expressed in micrograms per deciliter (ug/dL). Three participants were missing one or more cortisol samples, leaving 53 participants for analysis of variance (ANOVA) calculations and 54 participants for follow-up *t*-tests.

Salivary alpha-amylase (sAA) was measured using a kinetic reaction assay that employs a chromagenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose (Salimetrics, State College, PA; See Granger et al., 2006 for a detailed explanation). Intraassay variation (CV) computed for the mean of 30 replicate tests was less than 7.5%. Interassay variation computed for the mean of average duplicates for 16 separate runs was less than 6%. Salivary alpha-amylase data are expressed in micrograms per microliter (ug/mL). Two participants were missing one or more sAA samples leaving 54 participants for ANOVA and 56 participants for follow-up *t*-tests.

Cardiovascular measures—Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured using a Dinamap, automated, oscillometric blood pressure monitor (Critikon Inc., Tampa, FL). Participants were fitted with an appropriately sized cuff on their non-dominant arm. SBP and DBP are expressed in mm/Hg and HR in beats per minutes. The accuracy and reliability of blood pressure and heart rate measures in response to psychosocial stress in children and adolescents is well established (e.g., Matthews, Woodall, & Stoney, 1990).

Task-related affect measures—Self-reported affect was measured six times over the course of the stress sessions: once at baseline, once for each of the three stressors, and twice during the post-stress recovery. Participants were asked to respond according to how they were feeling *during* each task; rating the intensity of emotion-related adjectives (adapted from the State Trait Anxiety Inventory-Child Version; Spielberger, 1973) along 3-point Likert scales. Affect measures included emotion faces at the extremes of each adjective's scale to assist children in anchoring high and low levels of each emotion. Emotion adjectives included: “nervous,” “sad,” “scared,” “upset,” “relaxed,” and “happy.”

Behavioral and emotional measures—*Child Behavior Checklist 6-18 (CBCL)* is a parent-report measure used to assess adjustment for both children and adolescents (Achenbach, 1991; Achenbach & Rescorla, 2001). The CBCL requests ratings for 113 behaviors along a 3-point Likert scale (0 = “not at all true,” to 2 = “very true”). The CBCL yields eight problem behavior subscales: Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Rule-Breaking Behavior and Aggressive Behavior; and two broadband scales of Internalizing Problems (i.e., Anxious/Depressed, Withdrawn/Depressed and Somatic Complaints) and Externalizing Problems (Rule-Breaking Behavior and Aggressive Behavior). Test-retest reliability ($r_s = .91$ and $.92$) and internal consistency (α 's = $.90$ and $.94$) for the Internalizing and Externalizing broadband scales are excellent (Achenbach & Rescorla, 2001). Similarly, the Total Problems composite ($r = .94$, $\alpha = .97$) and 8 subscales ($r_s = .82 - .92$, $\alpha_s = .78 - .94$) have shown good reliability and internal consistency (Achenbach & Rescorla, 2001). Due to restricted range of the *T*-score distributions and the non-clinical nature of this sample, raw CBCL scale scores, rather than *T*-scores, were utilized in all analyses. Ten participants were missing CBCL data, leaving 46 participants for all CBCL analyses.

The *Revised Children's Manifest Anxiety Scale (RCMAS)* (Reynolds & Richmond, 1978, 2000) is a self-report of measure of anxiety for children and adolescents aged 6 to 19. The 37-item, “yes-no” response measure results in a total anxiety score as well as four subscale scores: Physiological Anxiety (e.g., “Often I have trouble getting my breath”), Worry/Oversensitivity (“I worry a lot of the time”), Social Concerns/Concentration (“I feel that others do not like the way I do things”), and a Lie Scale (“I am always good”). The composite scale ($\alpha = .82$) and subscales ($\alpha_s = .64 - .76$) have shown good internal consistency (Reynolds & Richmond, 1985). Likewise for the current study internal consistency for the full scale is good ($\alpha = .82$) but varies for the subscales ($\alpha_s = .63$ for physiological to $.81$ for worry/oversensitivity). Two participants were missing RCMAS data, leaving 54 participants for all RCMAS analyses.

Data Transformation

Analyses for salivary cortisol, sAA, and cardiovascular measures were based on six matched time points. Time points were selected to optimize timing for cortisol (20-30 minutes after onset of each stressor (Dickerson & Kemeny, 2004); sAA (5-10 minutes after onset of each stressor; Nater et al., 2006), and cardiovascular (concurrent with each stressor; Matthews et

al., 1990) assessment relative to onset of stressors (see Stroud et al., 2009 for expanded description)

Distribution analyses revealed three cortisol and four sAA samples with outlying values (3+ SD's from the mean). Each outlier was transformed (winsorized) to equal the highest respective cortisol or sAA level below 3 standard deviations from the mean (Wilcox, 1994). After winsorizing, cortisol and sAA levels were normally distributed.

For all analyses, baseline cortisol was defined as the minimum of two baseline samples (which were highly correlated, $r = .79$, $p < .001$), and baseline sAA was defined as the first baseline sample (due to faster increases in sAA than cortisol following onset of stress). Peak sAA and cortisol were calculated as the maximum value achieved during or following the stressors. For cardiovascular variables, task-stress levels were the average of each measure (SBP, DBP, or HR) during each stress task, and baseline levels, the average of cardiovascular measures during the initial baseline period. Peak cardiovascular measures were defined as the maximum average value attained during any of the three stress periods. Baseline affect for all affect adjectives was defined as affect level reported after the initial 15-minute rest period. Max affect was defined as the highest level reported across the three stressors. Percent change (also referred to as 'reactivity') for all variables was calculated by dividing the difference between maximum and baseline levels by the baseline.

For the behavioral measures the distribution across externalizing and total problem behaviors showed that there were one and two outliers respectively, with scores at or around three standard deviations from the respective means. These outliers were each winsorized to one standard deviation from the mean.

Analytical Strategy

Primary analyses employed a series of repeated measures ANOVAs to assess task-related change in affect, sAA, cortisol, and cardiovascular physiology over the six time points. Greenhouse-Geisser adjustments were made for significant departure from the sphericity assumption (Vasey & Thayer, 1987) and t-tests were conducted to examine differences between baseline and post-stress measures. Bivariate correlations were computed to assess relations between sAA and the other physiological measures and to examine preliminary associations with psychological variables. Finally, multiple regression analyses were conducted to determine if sAA reactivity acts as a moderator of the relationship between cortisol reactivity and internalizing or externalizing symptoms (e.g., anxiety, problem behaviors). For each of the regression models, sAA, then cortisol reactivity (centered at the respective means) were entered followed by a sAA reactivity by cortisol reactivity interaction term to test for moderation effects. Given potential influences of gender on stress response (Rausch, Auerbach, & Grambling, 2008; Stroud, Epel, & Salovey, 2002; Stroud, Papandonatos, Williamson, & Dahl, 2004) and because the larger study (citation removed) indicated that development (age/pubertal development) and stressor type (social rejection, performance) significantly influenced cortisol and sAA responses, all regression analyses controlled for sex, age and session type.

Results

Task-Induced Affect: Manipulation Check

Repeated measures ANOVAs examining affect across six time points confirmed that the stress tasks induced significant changes in subjective ratings over the course of the stress sessions. Specifically, positive affect declined ("happy", $F [3.73, 182.80] = 7.51$, $p < .001$; "relaxed", $F [3.59, 179.56] = 13.23$, $p < .001$), while negative affect increased ("nervous", $F [2.72, 138.89] = 25.06$, $p < .001$; "upset", $F [3.01, 153.41] = 3.20$, $p < .05$; "scared", $F [2.78,$

130.67] = 4.21, $p < .01$). Only sadness did not show significant change over the course of stress sessions.

Physiological Stress Reactivity: Time Course and Sensitivity to Stress

Salivary Alpha-Amylase: sAA levels increased significantly over the course of the stress session (collapsing across stressor type), $F(3.24, 165.18) = 11.61, p < .001$. Follow-up paired sample t -test analysis indicated significant increases from baseline to peak sAA levels, $t(55) = 8.18, p < .001$. Additionally, 47 of 56 participants displayed a sAA increase of 10% or greater over their baseline levels ($M = 54\%$ increase). See Figure 1.

Salivary Cortisol: We also found a significant increase in cortisol levels over the course of the stress session, $F(2.09, 104.63) = 4.40, p < .05$. Specifically, a significant increase was found between baseline and peak cortisol levels, $t(53) = 4.42, p < .001$. Of 54 participants with complete cortisol data, 36 displayed an increase of 10% or greater over baseline ($M = 46\%$ increase). See Figure 2.

Cardiovascular Physiology: Cardiovascular measures also showed significant increases over the course of the stress session (SBP: $F[3.63, 174.09] = 18.14, p < .001$; DBP: $F[3.71, 181.85] = 19.79, p < .001$; HR: $F[3.59, 175.75] = 5.93, p < .001$). Further analyses revealed significant increases between baseline and peak levels for SBP, DBP, and HR, $t(55) = 13.55, p < .001$; $t(55) = 13.07, p < .001$; and $t(55) = 13.62, p < .001$, respectively. Of 55 participants with cardiovascular data, all but one displayed increases in SBP, DBP, and HR.

Associations between sAA, Cortisol and Cardiovascular Measures—As hypothesized, there was a significant positive association between sAA and SBP and HR reactivity, such that change in sAA from baseline to maximum levels was positively related to change in SBP, and HR (see Table 1). However, there was no significant association between sAA and DBP reactivity.

A slightly different pattern emerged for cortisol and cardiovascular measures. Cortisol reactivity was positively related to both SBP and DBP reactivity, r 's (54) = .30 and .28, respectively, p 's $< .05$, but not to HR reactivity ($r(54) = .14, ns$). sAA and cortisol reactivity were not significantly related to each, nor were these measures related at baseline or peak.

Biobehavioral Associations

Child Behavior: Parent report of child behavior on the CBCL indicated that mean internalizing, externalizing and total behavior problems were within normal ranges. Consistent with previous studies parents reported more total behavior problems and externalizing behaviors for boys than for girls.

In bivariate analyses, neither sAA nor cortisol or cardiovascular reactivity were associated with internalizing, externalizing or total behavior problems when examined individually (see Table 2). However, regression analysis revealed a significant interaction between sAA and cortisol reactivity in the prediction of total child behavior problems after controlling for sex, age, and session type. When total behavior problems was regressed on sAA and cortisol reactivity and their interaction term, there was a significant interaction between sAA and cortisol reactivity, accounting for a total of 18.2% of the variance in total behavior symptoms (Adj. $R^2 = .25$; $R^2\Delta = .12, p < .02$; unstandardized $b = -13.14$, 95% CI: -24.32 to -1.96). This significant interaction was deconstructed using procedures described by Aiken and West (1991). As shown in Figure 3, the slope of the relation between cortisol reactivity to the stressor tasks and total child behavior problems was plotted at 1 SD above and below the mean for sAA reactivity ($M = .54$; $SD = .47$). Among participants who displayed reduced

sAA reactivity (1 SD below the mean), there was a significant positive association between cortisol reactivity to the stressors and CBCL behavior problems, $b = 10.91$, $t = 2.28$, $p < .05$, such that greater cortisol reactivity was associated with a greater total number of problem behaviors. However, the association between cortisol reactivity and problem behaviors was non-significant for participants who displayed strong sAA reactivity to the tasks (1 SD above the mean).

Further examination indicated that both the interactive effects of sAA and cortisol reactivity associated with the CBCL total score were largely attributed to the Attention Problems and Social Problems subscales. This model accounted for 26.8% of the variance in Attention Problems (cortisol: unstandardized $b = 1.44$, $p < .05$; interaction: unstandardized $b = -3.54$, $p < .01$) and 24.6% of the variance in Social Problems (cortisol: unstandardized $b = 1.04$, $p < .05$; interaction: unstandardized $b = -2.12$, $p < .01$). Deconstructing these interactions revealed similar associations to those observed for total behavior symptoms. Cortisol reactivity was found to be positively associated with attention and social problems for those individuals who showed little or no sAA reactivity to the task series (p 's $< .01$).

Contrary to hypothesis, we found no main effect or interaction of sAA and cortisol reactivity in the prediction of externalizing behaviors, however, we did find an interaction effect in the prediction of internalizing symptoms. Cortisol was positively associated with internalizing symptoms, but only in the context of low sAA. There was also a trend towards a positive main effect of cortisol. The model accounted for 20.8% on the variance in internalizing symptoms (cortisol: unstandardized $b = 3.92$, $p < .06$; interaction: unstandardized $b = -4.75$, $p < .05$). The interaction of cortisol and sAA in predicting internalizing symptoms was largely due to its association with the anxiety and depression symptom subscale (interaction: unstandardized $b = -2.29$, $p < .05$).

Anxiety: To further assess the relations between sAA and cortisol in relation to child behaviors and symptoms, correlation and regression analyses were conducted in the prediction of child-reported anxiety as measured by the RCMAS. Child-reported anxiety was associated with sAA response to the stressor tasks. RCMAS composite scores were positively associated with both baseline, and maximum sAA levels, but not with sAA reactivity (Table 2). In particular, baseline sAA levels were significantly associated with the Worry/Oversensitivity subscale, $r(52) = .28$, $p < .05$, while maximum sAA levels were positively associated with Physiological Anxiety, $r(52) = .27$, $p < .05$. In contrast, there were no associations between cortisol levels, heart rate, or blood pressure changes with self-reported anxiety as indexed by the RCMAS. Regression analyses did not reveal a moderating relationship in the prediction of child anxiety with or without sex, age and session type in the model.

In accordance with the finding that RCMAS composite was not significantly associated with sAA reactivity or other physiological reactivity, exploratory analyses of physiological associations after accounting for anxiety, revealed no significant changes from the associations reported earlier.

Discussion

This study produced several confirmatory findings and extends information regarding the biobehavioral associations among sAA, salivary cortisol, and internalizing symptoms. Our findings confirmed previous observations that sAA is more responsive than cortisol to laboratory stressors, as well as provided corroborating evidence for distinct kinetic response profiles for sAA and cortisol stress-related reactivity and recovery (Gordis et al., 2006; Nater et al., 2006). Consistent with a large literature on timing of ANS versus HPA response

(e.g., Chrousos & Gold, 1992), the profile of the sAA response rapidly reached a peak and recovered more quickly than did the response profile for cortisol. Also confirming prior work with adults (West, Granger, Kivlighan, Psota, & Hurston, 2006) and youth (Kivlighan, Wewerka, Gunnar, & Granger, 2006), individual differences in sAA were associated with cardiovascular physiology. Most noteworthy, we found evidence that asymmetry between ANS and HPA reactivity is related to both total behavioral problems and internalizing symptoms, with links between cortisol and behavior symptoms emerging only in the context of low sAA reactivity. In addition, we found a positive association between baseline sAA and children's report of anxious affect. Taken together, these findings support mounting evidence that individual differences in sAA reflect ANS activity. The findings have several notably implications.

Salivary Alpha-Amylase and Cortisol: Distinct Reactivity Profiles and Correlates

As expected, sAA and cortisol levels increased significantly over the course of the stressors, with significant increases occurring from baseline to peak. Consistent with previous findings (Chatterton et al., 1996; Granger et al., 2006; Nater et al., 2006), sAA displayed a faster response to stress, peaking 10 minutes earlier than cortisol and approximately 10 minutes after the onset of each stressor. Cortisol and sAA are distinct markers of stress reactivity; no significant correlations were found between sAA and cortisol either at baseline or when examining percent change from baseline to peak. sAA and cortisol were also differentially associated with cardiovascular measures. Changes in sAA and cortisol were both positively associated with changes in SBP; however, only sAA reactivity was associated with changes in HR, and only greater cortisol reactivity was linked to greater DBP reactivity. Interestingly, low baseline sAA was associated with *greater* heart rate reactivity, indicating that low *resting* levels of sAA may be predictive of a stronger cardiovascular reactivity to stress. These findings support the assumption that sAA is a surrogate marker of SNS activity and regulation.

Symmetry between ANS and HPA Reactivity: Links to Parent Reported Problem Behaviors

Following Bauer et al. (2002), this study examined whether HPA reactivity and ANS reactivity, and interactions between these systems, were associated with children's behaviors. "Asymmetry" between ANS and HPA reactivity was related to attention and social problems, as well as to anxious/depressed symptoms with associations between higher cortisol reactivity and adjustment problems, emerging only in the context of low sAA reactivity. Contrary to expectation, we did not find a similar pattern of asymmetry in relation to parental report of externalizing (e.g., aggression, rule breaking) behaviors in this sample of healthy, non-referred children and adolescents. However, both attention problems and social behavior problems might serve as precursors to more problematic externalizing behaviors, such as the aggressive behaviors reported among Gordis et al.'s (2006) sample of 10 to 14 year old, ethnically diverse, maltreated and non-maltreated youth. In Gordis et al.'s study, an interaction between sAA and cortisol reactivity predicted parent-rated aggression, a more circumscribed externalizing spectrum behavior. However, in contrast to our finding that high cortisol reactivity predicted problem behaviors in the context of low sAA reactivity, Gordis et al.'s (2006) study of both maltreated and non-maltreated youth found that low cortisol predicted aggressive behaviors in the context of low sAA reactivity. Although the patterns of cortisol were inconsistent across studies, the context of low sAA is consistent with previous findings of autonomic underarousal in both attention problems (van Lang et al., 2007) and aggressive behaviors (see Ortiz & Raine, 2004). Interestingly, the interaction effect of low sAA with cortisol was not found for healthy, nonreferred younger children (mean age of 8) studied by El-Sheikh, Erath, Buchalt, Granger and Mize (2008). El-Sheikh et al. (2008) found that basal cortisol levels were positively associated with both externalizing and internalizing behaviors in the context of high basal sAA.

Together, these findings suggest that future studies should focus on the role of sAA and cortisol asymmetry in the prediction of dimensions of externalizing behaviors (e.g., from attention problems to proactive aggression) within one sample. Studies must also integrate the complexities of age, gender, ethnicity, and life experiences in the prediction of these externalizing spectrum behaviors. As an example of the intricacies associated with SNS and HPA prediction of child and adolescent behaviors, Gordis, Granger, Susman and Trickett's (2008) follow up of Gordis et al.'s (2006) study found a significant interaction effect between the experience of child maltreatment with both basal and reactive sAA levels in the prediction of cortisol. An overall lack of correlation was found between sAA and cortisol among maltreated youth, but not among the comparison group. The researchers concluded that sAA and cortisol asymmetry was consistent among the subsample of maltreated youth, but not among the comparison group (Gordis et al., 2008), indicating that patterns of sAA and cortisol interaction might be highly dependent on individual characteristics, including maltreatment experiences.

Salivary Alpha-amylase and Trait Anxiety

In keeping with our examination of individual differences in the association between ANS and HPA activity, this study sought to explore the role of anxiety in children and adolescents' response to stress. Trait-anxiety was related to sAA changes, yet not to other measures of physiological reactivity. Children and adolescents reporting high levels of trait-anxiety exhibited lower sAA change in response to stress. In contrast, baseline sAA was *positively* associated with trait-anxiety, with higher baseline sAA predicting higher anxiety. In other words, children reporting high anxiety exhibited high levels of sAA at baseline and showed little sAA increase in response to stress, whereas low-anxiety children displayed low levels of baseline sAA, and displayed greater sAA reactivity. It is possible that the stressors used in this study were unable to evoke greater incremental stress for "high-stressed" children, but were perceived as "sufficiently stressful" to "low-stressed" children. A similar ceiling effect for sAA activity was found in a study of Generalized Anxiety Disorder (GAD) among college students (Fisher et al., 2010), whereby higher sAA at baseline was associated with lesser sAA change. This attenuated change was significantly more pronounced for students meeting criteria for GAD versus healthy controls and students with comorbid GAD and other disorders. Such a steady state of ANS arousal for youth with high-anxiety might have implications for long-term health and behavioral functioning. sAA may offer opportunity for assessment of ANS functioning under normative stress environments, such as during classroom (versus laboratory) academic challenges. Notably, neither cortisol nor cardiovascular measures were able to detect significant differences between "high-stressed" and "low-stressed" participants, further highlighting the potential for sAA's unique contribution as a measure of stress reactivity as well as a potential measure of chronic stress and anxiety.

While the present study expands the knowledge in the field with respect to sAA activity in children and adolescents, there are several limitations. First, the majority of participants in this sample of healthy children were Caucasian and from middle-to-upper class households. Although homogeneity of the sample was important in this early study of a novel surrogate biomarker, findings must be interpreted with caution. Both race and socio-economic status have been previously identified as variables potentially mediating the stress response in youth (Barnes et al., 2000; Lupien, King, Meaney, & McEwen, 2001). Thus, future studies with more diverse samples are needed.

A second limitation is the small sample size and thus, the need to collapse across gender, age, and stressor type in analyses to determine associations between cortisol/sAA reactivity and behavioral and emotional functioning. However, that associations emerged even after controlling for gender, development, and stressor type, suggests that effects may be even

stronger in a larger sample. Future studies might examine differential associations between cortisol/sAA reactivity and behavioral and emotional symptoms by age, gender, and particularly, stressor type. A final limitation is the limited range of behavioral and emotional symptoms among the sample. Although inclusion of healthy children was important to the study design, limited variance in CBCL and RCMAS scores might have increased the risk of Type II errors. Again, the fact significant interactions between HPA and SNS systems emerged in this small sample with limited variability in behavioral and emotional symptoms highlights a rich and exciting area for future inquiry.

Conclusions

The finding that sAA and cortisol interact to predict child adjustment supports theory (Bauer et al., 2002) and builds on an emerging literature linking patterns of physiological responses among multiple stress systems, to child functioning. However, as noted by El-Sheikh and colleagues (2008), integration of two biological subsystems has added complexity. The ANS and HPA differ in terms of their response timing; sensitivity to different aspects of stress; dependence on age, gender, ethnicity, and social-contextual factors; as well as habituation to repeated exposure to the same events. However, ideally, elucidating complex interactions among multiple biological systems and psychological mechanisms will lead to a more accurate and multifaceted vision of the development of human behavior.

Acknowledgments

This research was supported in part by an NIMH Career Development Award (K23 MHXXXX), a National Alliance for Research on Schizophrenia and Depression Junior Investigator Award to the last author, and NCI grant P50 CAXXXX. Further acknowledgements removed.

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Highlights

sAA activity is related to ANS activity but not to HPA activity

In youth, sAA is associated with systolic blood pressure and heart rate reactivity

sAA moderates the association between cortisol and problem behaviors

Figure 1. Amylase Over Time

Figure 2.
Cortisol Over Time.

Figure 3.
Cortisol reactivity is associated with total behavior symptoms only for those that display little or no sAA reactivity to the stressor tasks.

Table 1
Correlations between Salivary Alpha Amylase and Physiological Measures

	Salivary Alpha Amylase		
	Baseline	Maximum	% Change
<u>Baseline</u>			
1. Cortisol	-.15	.02	.25
2. SBP	.12	.17	.18
3. DBP	.02	.09	.28*
4. HR	.13	.21	.30*
<u>Maximum</u>			
5. Cortisol	-.16	-.06	.18
6. SBP	.02	.18	.38**
7. DBP	-.02	.11	.33*
8. HR	-.10	.10	.49***
<u>Percent Change</u>			
9. Cortisol	-.13	-.10	.10
10. SBP	-.14	.05	.39**
11. DBP	.04	.06	.16
12. HR	-.26*	-.10	.27*

*
 $p < .05$

**
 $p < .01$

 $p < .001$

Table 2
Correlations between Physiological and Behavioral Measures

	CBCL Total ^a (<i>M</i> = 14.87, <i>sd</i> = 12.88)	Externalizing ^b (<i>M</i> = 4.85, <i>sd</i> = 4.45)	Internalizing (<i>M</i> = 4.98, <i>sd</i> = 5.29)	Anxiety (<i>M</i> = 7.82, <i>sd</i> = 5.54)
Baseline				
sAA	.20	.18	.16	.35**
Cortisol	.04	.01	.09	-.10
SBP	.05	-.12	.14	.04
DBP	.03	-.14	.18	-.02
HR	.08	-.10	.17	-.07
Maximum				
sAA	.14	.08	.18	.28*
Cortisol	.25⁺	.10	.30*	-.08
SBP	-.04	-.15	.06	.08
DBP	.08	-.06	.18	.10
HR	.04	-.08	.16	.09
Percent Change				
sAA	-.09	-.19	.08	-.09
Cortisol	.16	.03	.17	-.04
SBP	-.12	-.08	-.09	.09
DBP	.06	.04	.05	.16
HR	-.05	.00	.00	.20

⁺ $p < .10$

^{*} $p < .05$

^{**} $p < .01$

^{***} $p < .001$

^a $M = 15.54$, $sd = 15.25$, before winsorizing;

^b $M = 5.13$, $sd = 5.68$ before winsorizing

Note: All mean behavior and symptom scores are within normative ranges