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Child depressive symptoms: Associations with salivary cortisol and alpha amylase in two distinct challenges

Jennifer E. Khoury[a,*], Brittany Jamieson[b], Andrea Gonzalez[a], Leslie Atkinson[b]

[a] Department of Psychiatry and Behavioral Neurosciences, McMaster University, Offord Centre for Child Studies, 1280 Main Street West, Hamilton, Ontario, Canada
[b] Department of Psychology, Ryerson University, 350 Victoria Street, M5B 2K3, Toronto, Ontario, Canada

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ABSTRACT

Child depression has been inconsistency linked to cortisol and salivary alpha-amylase (sAA) reactivity. This study assessed three factors that contribute to inconsistencies: 1) the differential effectiveness of laboratory challenges to elicit cortisol and sAA; 2) the impact of cortisol-sAA coordination; and 3) variation in parent versus child ratings of depression. A community sample of 52 children participated in the Trier Social Stress Test-Child Version (TSST-C) and a competition challenge. Saliva was collected and assayed for cortisol and sAA. Analyses were conducted using multilevel modeling. Child-reported depressive symptoms were associated with a declining cortisol trajectory in the TSST-C. Mother-reported depressive symptoms were associated with higher baseline sAA in the TSST-C and the competition challenge. Further, child-reported depressive symptoms were associated with cortisol-sAA coordination in the competition challenge. Findings underscore the nature of the challenge and the behavioral informant as impacting associations between child depressive symptoms and cortisol and sAA secretion.

1. Introduction

Stress physiology plays an essential role in nearly all human physical and mental health processes (Reynolds, 2013) across the lifespan (Meaney & Szyf, 2005). The Hypothalamic-Pituitary-Adrenal (HPA) axis, including the production of the hormone cortisol, is a primary biological stress pathway. Depressive symptoms and broader internalizing behaviors (i.e., depression, anxiety, somatic symptoms) have been associated with both higher cortisol (Booij, Bouma, de Jonge, Ormel, & Oldehinkel, 2013) and lower cortisol reactivity (Harkness, Stewart, & Wynne-Edwards, 2011). In addition, the HPA axis response to stress is coordinated with the autonomic nervous system (ANS). An ANS marker, salivary alpha-amylase (sAA), has been shown to interact with cortisol to predict broader internalizing behaviors (El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008). The current study seeks to elucidate associations between depressive symptoms and cortisol and sAA reactivity, as well as coordination between the cortisol and sAA, by examining: 1) two different challenges to elicit stress responses, and 2) the informant (child or parent) of depressive symptoms.

1.1. Depression, cortisol and sAA activity, and developmental differences

Individuals with depressive symptoms have altered HPA axis activity. The majority of the adult literature suggests that depression is associated with higher cortisol reactivity (e.g., Ehlert, Gaab, & Heinrichs, 2001; Stetler & Miller, 2011). However, some studies show that atypical forms of depression (including mood reactivity, hyperphagia, hypersomnia, fatigue, and rejection sensitivity; Asnis, McGinn, & Sanderson, 1995) and depression in the context of life stressors are associated with blunted cortisol reactivity (Edwards, Heyman, & Swidan, 2011; Heim, Ehlert, & Hellhammer, 2000; O'Keane, Frodl, & Dinan, 2012). A number of factors, such as the time of onset, severity, and type of depression, likely impact these distinct associations.

Developmental psychopathology models highlight the importance of psychosocial and physiological stress in the etiology of depressive symptoms and internalizing disorders (e.g., Cicchetti & Toth, 1991; Guerry & Hastings, 2011). Most of this research focuses on adolescent samples, because base rates of depressive symptoms are low in childhood (Son & Kirchner, 2000). Despite low base rates, it is important to examine factors related to the development of depressive symptoms in childhood, as child depressive symptomatology is associated with increased risk of suicide and the continuation of depressive symptoms later in life (Fombonne, Wostear, Cooper, Harrington, & Rutter, 2001; Garber, Kriss, Koch, & Lindholm, 1988; Kovacs et al., 1984), resulting in additional social and economic burden (McCrone, Knapp, & Fombonne, 2005).

* Corresponding author at: Department of Psychiatry and Behavioral Neuroscience, Offord Centre for Child Studies, Canada
E-mail address: jennifer.e.khoury@gmail.com (J.E. Khoury).

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In order to understand the pathophysiology of depression, the normative developmental changes in cortisol and sAA reactivity from childhood to adolescence must be considered. Infancy and much of childhood has been proposed as a relative period of physiological hyporeactivity (Gunnar & Quevedo, 2007). Indeed, children demonstrate lower cortisol and sAA reactivity compared to adolescents (Granger et al., 2006; Gunnar, Talge, & Herrera, 2009; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Stroud et al., 2009). This supports a normative developmental trend towards increased stress reactivity from childhood to adolescence. While taking into account these normative developmental changes, we aim to clarify the interplay between stress physiology and child depressive symptoms, within different stressful contexts.

1.2. Associations between depression, cortisol and sAA reactivity in different contexts

Stressor paradigms vary in their capability to evoke stress reactivity (e.g., Dickerson & Kemeny, 2004; Gunnar, Talge et al., 2009; Jansen, Beijers, Riksen-Walraven, & de Weerth, 2010). It is theorized that the HPA axis and ANS activity are triggered by different contexts. Research indicates that the HPA axis responds most strongly to socially threatening, unpredictable and uncontrollable stressors (Dickerson & Kemeny, 2004; Jansen et al., 2010). For example, for adolescents, cortisol is elevated in peer-rejection challenges (Stroud et al., 2009) and social-evaluative contexts (i.e., the Trier Social Stress Test, TSST, Kirschbaum, Pirke, & Hellhammer, 1993), though cortisol is more pronounced in the latter. However, research is less conclusive regarding the type of challenges associated with sympathetic ANS activation. Theories suggest that the sympathetic branch of the ANS responds strongly to general challenge (Frankenhaeuser, Lundberg, & Forsman, 1980; Nigg, 2006), including effortful and controllable challenges (Flinn, 2006; Frankenhaeuser et al., 1980; Laurent, Ablow, & Measelle, 2012; Lundberg & Frankenhaeuser, 1980; Peters et al., 1998). Indeed, infants showed higher sAA in response to an effort-based (clean-up) challenge compared to an attachment challenge (Laurent et al., 2012). Only one study to date has examined cortisol and sAA responses to four different challenges, demonstrating that the highest cortisol response was evoked by the TSST, whereas the highest sAA response was evoked by the physical exercise ergometer test (Skoluda et al., 2015). Thus, the type of challenge itself is a predictor of cortisol and sAA reactivity.

In addition, the association between depression and cortisol reactivity differs by the paradigm used to elicit stress. Adolescents with high self-reported depressive symptoms and internalizing behaviors demonstrate blunted cortisol during the TSST-C (Booij et al., 2013; Harkness et al., 2011), a conflict discussion task (Spies, Margolin, Susman, & Gordis, 2011), and other mildly stressful tasks (Suzuki, Belden, Spitznagel, Dietrich, & Luby, 2013), though the same pattern has not been found in other studies (Granger, Weisz, & Kauneckis, 1994; Steeger, Cook, & Connell, 2016). To date, one study has examined the association between internalizing behavior and cortisol reactivity in two distinct challenges, in a sample of 8 to 12-year-olds. Laurent, Vergara-Lopez, and Stroud (2016) found that maternal-reported internalizing behavior was not associated with cortisol reactivity in a performance challenge; however internalizing behaviors predicted an earlier cortisol peak and a less dynamic overall response curve in a peer-rejection challenge. Taken together, these findings indicate that internalizing behaviors are differentially associated with cortisol reactivity in distinct contexts.

Given the extensive physiological changes that occur during puberty (Angold & Worthman, 1993), there are age-related differences in the association between depression and cortisol reactivity across contexts. For example, third and sixth graders with elevated dysphoric symptoms demonstrated a declining cortisol response to a psychosocial challenge consisting of a conflict discussion and a video-recorded verbal speech, whereas ninth graders demonstrated an increasing cortisol response to this challenge (Hankin, Badanes, Abela, & Watamura, 2010). In a longitudinal study of 10 and 12-year-olds, higher depressive symptoms were associated with lower cortisol reactivity in the Cold-Pressor Task, but only in 12-year-olds (Keenan et al., 2013). Collectively, this research indicates that the link between depression and cortisol differs by age and is related to the paradigm used to induce stress.

In contrast to the vast literature associating cortisol to depressive symptoms, sAA has only recently emerged as a potential biomarker of adult depression (Schumacher, Kirschbaum, Fydrich, & Ströhle, 2013; Tanaka et al., 2012). However, researchers have not found a significant effect of child internalizing symptoms on sAA trajectories across divergent stressor paradigms (Bae et al., 2015; El-Sheikh et al., 2008; Koss et al., 2014). Importantly, research has not yet examined whether differentially stressful paradigms impact the associations between sAA and child depression/internalizing symptoms. In the current study, we compared a social-evaluative task to a novel competition challenge that evoked effort but not perceived-evaluation or uncontrollability. Our use of two distinct challenges may reveal unique associations between depressive symptoms, cortisol, sAA, and cortisol-sAA coordination.

1.3. Cortisol-sAA coordination

The concurrent examination of cortisol and sAA is vital for understanding the pathophysiology of depressive symptoms. The ANS system produces rapid and short-lasting effects, with peak sAA levels 5–10 minutes post-stress (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2008; Nater et al., 2005, 2006), compared to the slower-acting HPA axis, with peak cortisol levels 20–40 minutes post-stress (Dickerson & Kemeny, 2004). Coordination between the HPA axis and ANS can be excitatory or antagonistic (Atkinson, Jamieson, Khoury, Ludmer, & Gonzalez, 2016 for discussion; Sapolsky, Romero, Munck, 2000). Bauer, Quas, and Boyce (2002) posit that systems can be coordinated in either an *additive* or *interactive* manner. The *additive* model suggests that asynchronous activity (e.g., high activity in one system and low in the other) is optimal, whereas the *interactive* model suggests that synchronous activity in both systems is most adaptive (Bauer et al., 2002).

Importantly, cortisol-sAA coordination varies by the type of paradigm used to elicit stress. Using an amalgamation of challenges, El-Sheikh et al. (2008) found that children who had higher cortisol and higher sAA experienced greater internalizing behaviors (*synchronous additive coordination*). Koss et al. (2014) found that children in a high family conflict environment who evince low cortisol and high sAA levels in response to a conflict vignette challenge experienced the most internalizing behaviors (*asynchronous interactive coordination*). In contrast, additional research has not found significant coordination effects of cortisol, sAA, and internalizing behaviors (Chen, Raine, & Granger, 2015; Vigil, Geary, Granger, & Flinn, 2010). The divergent challenges used across these studies likely contributes to the differing findings.

1.4. Differences in parent and child report of internalizing behavior

Another important factor to consider is the informant of depressive symptoms. There is a low correlation (typically falling in the 0.20 s) between parent and child reports of psychopathology (Achenbach & Rescorla, 2001; De Los Reyes & Kazdin, 2005). Yet both reports represent valid, though unique, perspectives on child difficulties and are differentially related to stress physiology. For example, when examining the separate effects of parent- and self-reported difficulties, adolescent-reported, but not parent-reported, internalizing behavior predicted cortisol reactivity (Hartman, Hermanns, de Jong, & Ormel, 2013).

1.5. The current study

This study aimed to 1) examine associations between depressive

symptoms and cortisol and sAA reactivity, and 2) assess associations between depressive symptoms and cortisol-sAA coordination, in a sample of 8 to 10-year-old children. To this end, we employed several methodological controls: 1) use of two challenges, specifically designed to differentially elicit cortisol and sAA reactivity; 2) measurement of cortisol and sAA trajectories during these challenges; and 3) collection of parent and child reports of depressive symptoms. We hypothesized that child depressive symptoms will be associated with lower cortisol in the TSST and competition challenge. Given the inconclusive nature of prior sAA research, we did not have a priori hypotheses for the effects of depression on sAA or sAA-cortisol coordination across challenges. To address gaps and inconsistencies in the literature, this is the first study to assess childhood depressive symptoms in relation to cortisol, sAA, and cortisol-sAA coordination, in two distinct challenges, which were designed to differentially elicit cortisol and sAA.

2. Methods

2.1. Participants

Participants were mother-child dyads (N = 52) who were part of a larger longitudinal study (N = 314) (Atkinson et al., 2013). Participants were originally recruited from postings, community centers, and baby conventions in the Toronto area. Inclusion criteria at the onset of this longitudinal study were that infants were healthy with no major developmental disorder, pregnancy was over 32 weeks, and that mothers were 18 years or older at childbirth, had no known hormonal disorders, and were fluent in English. Families from the larger longitudinal study who had children between the ages of 8.5 and 10 years old (n = 114) were contacted and asked to participate in the follow-up study. The current sample did not differ from the larger longitudinal sample on demographic variables.

Children ranged from 8.5 to 10 years of age (M = 8.88, SD = 0.70). The sample included 29 male children (55.7 %). BMI ranged from 10.2 to 25.3 (M = 16.92, SD = 3.09), sex and age-adjusted height ranged from the 0th to 90th percentile (M = 16.44, SD = 23.02), and weight ranged from the 1st to 99th percentile (M = 58.68, SD = 32.25). The majority of the sample was Caucasian (76.5 %), with a smaller proportion of Asian (9.8 %), Afro-Canadian (2.0 %) and 'mixed' ethnicities (11.8 %). Children were primarily from middle to high socio-economic backgrounds (69.5 % family income > $100,000). Five children were taking medication/supplements, including iron supplements (n = 1), melatonin (n = 1), and stimulant medication (Concerta n = 1, Strattera n = 1, Biphentin n = 1). In total, 46 children completed both study sessions; five families could not be reached to schedule the second session, and one declined participation in the second session. Families who did not participate in the second study visit did not differ on key demographic characteristics. In addition, one child was excluded due to a diagnosis of Autism Spectrum Disorder, which prevented standard implementation of study procedures. In total, the final sample included 51 children.

2.2. Procedure

Mother-child dyads participated in two laboratory sessions, approximately three weeks apart (*Median* = 2.00 weeks, Interquartile Range = 2.00 weeks). At the beginning of each session dyads relaxed for 15 min, while two baseline saliva samples were collected (-15 min and 0 min). Immediately after the second saliva sample was taken (0 min), children participated in a 20-minute challenge (TSST-C or Competition) and mothers observed behind a one-way mirror. The order of the challenges was randomly counterbalanced (49 % completed the TSST-C first). Following the challenges, additional saliva samples were taken and dyads completed questionnaires. During the first study session only, depression measures were completed and children's weight and height were measured. Procedures occurred between 1300 h and 1800 h to control for circadian rhythm of cortisol and sAA.

2.3. Measures

2.3.1. The Trier Social Stress Test- Child Version (TSST-C)

The TSST-C is a social-evaluative, performance challenge adapted for children 7 years and older (Buske-Kirschbaum et al., 1997). The TSST-C began with the experimenter providing instructions and reading the child the beginning of a story in front of two expert judges (5 min), the child then prepared the rest of the story in a separate room (5-minute preparation), and then told the story in front of the two judges (5-minute speech). If a child finished their story before five minutes elapsed or did not speak for 20 s, a judge prompted them to continue. After completing the story, the child was instructed to serially subtract the number 7 from 758 as quickly as possible, without making mistakes (5-minute arithmetic task). Participants were asked to start over if errors were made. Children were told that the task would be video-recorded and later reviewed by the judges. Two confederates (a combination of different males and/or females) in laboratory coats acted as expert judges and displayed a neutral expression throughout the challenge.

2.3.2. Competition challenge

In the competition challenge, children completed an adapted 5-minute mirror tracing task (Bostock, Hamer, Wawrzyniak, Mitchell, & Steptoe, 2011; Hamer et al., 2006). During this activity children used a mirror tracing apparatus to trace the outline of a star, as quickly as possible, without making errors. This task was adapted so that the apparatus did not buzz when children made a mistake, so as to prevent interference with the amount of effort put forth in the challenge. The second part of the competition was a 100-piece puzzle to be completed within the 10-minute time limit. Children were told to work as quickly as possible to complete the puzzle and that the child who finishes the puzzle the fastest wins the competition. No participant completed the puzzle in the time provided (although all were given prizes). Except for times of instruction, children were left alone to complete this challenge.

2.3.3. Ethical considerations

Children and mothers were free to terminate procedures at any time. If children became noticeably upset during a challenge, they were asked if they wanted to continue. Ten and four children prematurely terminated the TSST-C and competition challenge, respectively (only one child terminated both challenges prematurely).[1] This rate of discontinuation is comparable to a similar-aged sample participating in the TSST-C (Tackett, Herzhoff, Smack, Reardon, & Adam, 2017). At the end of the study (i.e., after the last saliva sample is taken), complete disclosure was provided and children were offered an opportunity to meet the judges and receive positive feedback (TSST-C) and children were given positive feedback about their performance (Competition).

2.3.4. Saliva collection and assays

All participants refrained from drinking, eating, and brushing their teeth one hour before saliva sampling (Kirschbaum & Hellhammer, 1994, 1989). However, one child sipped water during the session due to hiccups. Saliva was collected using sorbettes (Salimetrics, State Collee PA). Cortisol was assayed using the -15, 0, +10, +20, +40, and +60 min samples, whereas sAA was assayed using the -15, 0, +5, +10, and +20 min samples, given the slower reactivity of cortisol compared

[1] Children who terminated the TSST-C or competition challenge prematurely did not differ in their cortisol or sAA levels from those who completed the challenges [cortisol: t(267) = -1.43, p = .15 and t(304) = 1.26, p = .21, sAA: t(247) = -.57, p = .57 and t(273) = .16, p = .88, for the TSST-C and competition challenge, respectively].

Table 1
Descriptive statistics.

	M	SD	Range
Cortisol TSST-C (log transformed, nmol/L)			
−15 min	0.53	0.28	−0.16 to 1.28
0 min	0.52	0.29	0.02 – 1.39
10 min	0.68	0.43	0.06 – 1.94
20 min	0.69	0.38	0.13 – 1.87
40 min	0.60	0.30	0.16 – 1.55
60 min	0.56	0.25	0.15 – 1.28
sAA TSST-C (log transformed, U/mL)			
−15 min	1.75	0.29	0.99 – 2.31
0 min	1.74	0.29	0.91 – 2.28
5 min	1.79	0.31	1.03 – 2.30
10 min	1.73	0.29	0.94 – 2.38
20 min	1.71	0.25	1.01 – 2.17
Cortisol COMP (log transformed, nmol/L)			
−15 min	0.47	0.24	−0.36 to 1.10
0 min	0.44	0.23	−0.17 to 1.16
10 min	0.38	0.27	−0.41 to 1.07
20 min	0.36	0.30	−0.43 to 1.04
40 min	0.41	0.24	−0.21 to 1.09
60 min	0.46	0.31	−0.28 –1.24
sAA COMP (log transformed, U/mL)			
−15 min	1.78	0.27	1.22 – 2.38
0 min	1.77	0.29	1.00 – 2.22
5 min	1.75	0.26	0.94 – 2.14
10 min	1.73	0.28	0.96 – 2.20
20 min	1.73	0.31	0.98 – 2.18
CDI self-reported depression	7.57	6.146	0–30
CBCL maternal-reported depressive symptoms	2.08	2.35	0–8

Note: TSST = Trier Social Stress Test- Child Version; COMP = Competition challenge (Puzzle/mirror tracing activities); sAA = salivary alpha amylase; CDI = Child Depression Inventory; CBCL = Child Behaviour Checklist.

to sAA (Dickerson & Kemeny, 2004; Nater & Rohleder, 2009). In the present data, cortisol peaked between 10 and 20 min. and sAA peaked at 5 min (Table 1 and Fig. 1).For the coordination analyses only, the cortisol 10 min sample was removed to match the cortisol 20 min and sAA 5-minute samples (respective peaks).

Sorbettes were placed in a 2-mL cryovial, sealed, and stored at -70 °C. Samples were thawed overnight in the refrigerator before they were assayed. Samples were centrifuged for 20 min at 3000 rpm at 4 °C for cortisol assays and for 15 min at 3000 rpm at 4 °C for alpha-amylase assays. Saliva was assayed using cortisol and alpha-amylase immunoassay kit (Salimetrics, State College, PA). Samples from each child across both sessions were assayed together. All samples were assayed in duplicate and average values were used in analyses. For cortisol, the inter-assay variation was 7.96 % and the intra-assay variation was 8.45 %. For sAA, the inter-assay variation was 9.43 % and the intra-assay variation was below 5 % for low, medium, and high samples.

2.3.5. Child-reported depressive symptoms

Child self-reported depressive symptoms were assessed using the 27-item Child Depression Inventory, Second Edition (CDI-2; Kovacs, 1992). The CDI-2 is a comprehensive measure of depressive symptoms, within the past two-weeks, for children and adolescents 7–17 years old. The CDI is a psychometrically sound measure, with high reliability and predictive validity in both community and clinical samples (Masip, Amador-Campos, Gómez-Benito, & del Barrio Gándara, 2010). The CDI had strong internal consistency in this sample (Chronbach's α = .82).

2.3.6. Maternal reported depressive symptoms

Using multiple informants is considered best practice when assessing child and youth psychopathology (Achenbach, 2009; Jensen et al., 1999). Thus, in addition to child-reported symptoms, mothers also completed the parent-version of the Child Behavior Check List for school-aged children, 6–18 years old (CBCL 6–18; Achenbach & Rescorla, 2001). In the current study, the 13-item DSM Affective Problems scale was used as an index of depressive symptoms. The Affective Problems scale had acceptable internal consistency (Chronbach's α = .67).

2.3.7. Demographics and covariates

Mothers reported demographic information, including the age, sex, BMI, and ethnicity of children, and family income. In addition, medication use, the time gap between sessions, time since waking, time of last meal, and confederate gender were noted as these variables might influence cortisol and sAA levels (e.g., Goldberg et al., 2003; Ruttle et al., 2013).

3. Results

3.1. Data preparation and analytic approach

Data were analyzed using multilevel modelling (MLM) with maximum likelihood (*Hierarchical Linear Modeling, Version 7,* Raudenbush, Bryk, Cheong, Congdon, & Du Toit, 2011) to account for interdependence of cortisol and sAA across time and within participants. MLM accounts for individual differences in initial cortisol and sAA values (i.e., intercepts) and change over time (i.e., slopes), and how these differ according to predictors of interest (Hruschka, Kohrt, & Worthman, 2005; Singer & Willet, 2003; Willett & Sayer, 1994). MLM is robust to missing data at lower levels (i.e., cortisol and sAA) and permits uneven spacing between samples (Hruschka et al., 2005; Laurent,

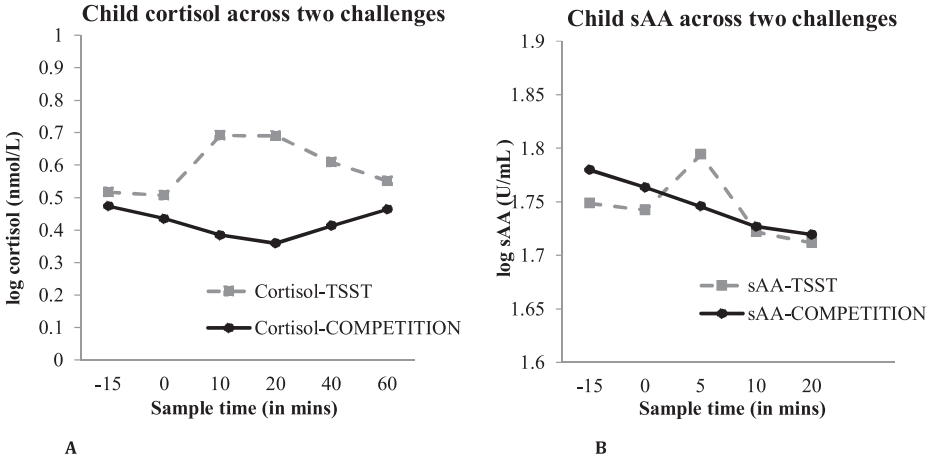

Fig. 1. Panel A depicts salivary cortisol trajectories across the Trier Social Stress Test (TSST) and puzzle/mirror challenge (COMPETITION). Panel B depicts salivary alpha amylase (sAA) trajectories across the Trier Social Stress Test-Child Version (TSST-C) and puzzle/mirror challenge (COMPETITION).

Table 2
Correlations between salivary cortisol, salivary alpha amylase, and internalizing predictors.

	1.	2.	3.	4	5.	6.
1. log Cortisol TSST	–					
2. log Cortisol COMP	.40**	–				
3. sAA TSST	-.10	-.10	–			
4. sAA COMP	-.09	.20**	.55**	–		
5. CDI	-.08	-.06	.25**	.21**	–	
6. CBCL Depression	-.24**	-.130*	.26**	.023	.24	–

Note: TSST = Trier Social Stress Test; COMP = Competition challenge (puzzle and mirror tracing activities); sAA = salivary alpha amylase (log transformed); CDI = Child Depression Inventory total score; CBCL = Child Behavior Checklist Internalizing subscale.
*p < .05, **p < .01.

Gilliam, Wright, & Fisher, 2015).

We conducted three sets of analyses with distinct predictors/outcomes: 1) depression predicting cortisol, 2) depression predicting sAA, and 3) depression predicting sAA-cortisol coordination. Two-level multilevel models were used to assess child cortisol and sAA trajectories across collection time (Level 1), nested within individual children (Level 2). Within each set of analyses, separate MLM models were conducted for each psychological predictor (i.e., child- and maternal-reported depressive symptoms), within each challenge (i.e., TSST-C and puzzle/mirror-tracing competition). Results are presented with time centered at baseline (i.e., time = 0). Analyses were re-run with time centered at peak cortisol (time = 20 and 5 for cortisol and sAA, respectively) to derive intercept and interaction effects at this specific point in the trajectory.

In the cortisol outcome models, Level 1 predictors included linear time (coded as -15, 0, 10, 20, 40, 60 min) and quadratic time (coded as 225, 0, 100, 400, 1600, 3600 min, time^2), directly corresponding to -15, 0, 10, 20, 40, and 60 min for cortisol. In the sAA outcome models, linear time was coded as -15, 0, 5, 10, 20 min and quadratic time was coded as 225, 0, 25, 100, 400. The exact time of saliva collection was entered into models, instead of estimated nominal time, in order to capture variability in sampling time between participants. To facilitate model convergence, all exact time values were divided by 10 (due to large values for quadratic time). For the coordination models, sAA was entered as a person-centered variable (sAA value at each time point minus the individual's mean across time points) at Level 1. Cortisol and sAA values were matched on time, such that cortisol samples at -15, 0, +20, +40, and +60 min were aligned with sAA samples at -15, 0, +5, +10, and +20 min. This allowed for the cortisol peak of 20 min to be matched with sAA peak of 5 min (see Table 1). Interaction terms between sAA and each time variable were also entered as Level 1 predictors. At Level 2, the relevant depression predictor (child or maternal report) was entered (grand mean centered) and two-way and three-way interactions between the Level 2 depression predictor and Level 1 predictors were entered.

A bottom-up, blocked approach, using full maximum likelihood estimation was used to derive a parsimonious model (McCoach, 2008). Blocks were tested in the following sequence: null model, Level 1 fixed effects, Level 1 random effects (i.e., slope of linear and quadratic time), Level 2 fixed effects and slope interactions, and covariates as appropriate. Decisions to include various components were based on (i) theoretical considerations, (ii) comparison of model fit using the log-likelihood ratio test, and (iii) whether a predictor significantly accounted for model variance (Raudenbush & Bryk, 2002). Final estimation without robust standard errors (given the small sample size) was used to interpret fixed effects. The matrix was set as a T distribution where the maximum distribution is [(Q + 1) x (Q + 1)], where Q is the number of level 1 coefficients. All final models were re-estimated using restricted maximum likelihood (REML) for a conservative estimation of variance-covariance structure.

3.2. Descriptive results and exploration of covariates

Cortisol and sAA values were log transformed to address positive skew. Outliers were tested using Mahalanbois distance (Hadi & Simonoff, 1993), a statistical procedure used to identify outliers in multivariate data. After log transformations, two cortisol outliers during the TSST-C remained significant. These extreme outliers (+3 SD) were modified using winsorization (Ghosh & Vogt, 2012). Descriptive statistics for log-transformed cortisol (nmol/L) and sAA (U/mL), as well as child- and parent-reported depressive symptoms are presented Table 1. Child self-reported depressive symptoms scores ranged from 0 to 30 (M = 7.57, SD = 6.15); 12 % of the sample reported CDI scores in the Clinical range, 8 % in the Borderline Clinical range, and 80 % in the Non-Clinical range. Maternal-reported depressive scores ranged from 0 to 8 (M = 2.08, SD = 2.35); 6 % of the sample reported depressive symptom scores in the Clinical range, 4 % in the Borderline Clinical range, 89 % in the Non-Clinical range. Correlations between cortisol, sAA, child- and maternal-reported depressive symptoms are shown in Table 2.

In regards to missing data in the TSST, 44 participants had complete cortisol data and 39 had complete sAA data (out of a possible 45). In the competition challenge, all 45 participants had complete cortisol data and 40 had complete sAA. All of the 45 participants who participated in both visits completed the CDI, whereas 44 completed the CBCL. Importantly, MLM accounts for all missing cortisol and sAA outcome data (Hruschka et al., 2005). More detailed information about missing data is included in Supplementary Table 1.

Socio-demographic factors, as well as time since waking and time of last meal, time between lab visits, and gender of the confederate judges in the TSST were explored as covariates in the final stage of MLM analyses (see Table 3 for preliminary correlations). To facilitate comparison of findings across challenges, common sets of covariates were included in models across challenges. More specifically, if a covariate was significant in either the TSST or puzzle challenge, it was retained in the final model for both challenges. Separate covariates were included in models with cortisol and sAA outcomes, given that these are different stress systems.

3.3. Cortisol and alpha amylase trajectories across challenges

Average salivary cortisol and sAA trajectories across time and challenge are depicted in Fig. 1 Panel A and B, respectively. Predictors of cortisol and sAA trajectories, in each challenge, are explored in

Table 3
Correlations between salivary cortisol and potential covariates.

	1. log Cortisol TSST	2. log Cortisol COMP	3. log sAA TSST	4. log sAA COMP
1. Child age	-.028	-.038	.250**	.140*
2. BMI	-.036	-.056	.090	.001
3. Medication	.059	-.023	.264**	.212**
4. Ethnicity	.024	.168**	.027	.228**
5. Family income	.062	.106	-.026	-.111
6. Last time ate	-.140*	-.304**	.044	-.226*
7. Time since waking	-.290*	-.330**	.074	-.126
8. Lab visit order	-.023	.181**	-.103	-.137*
9. Time between lab visits	-.079	-.280**	-.0001	.025
10. Sex	.156*	.034	-.125	-.084
11. Gender of confederate judges	-.067	n/a	.171*	n/a

Note: TSST = Trier Social Stress Test; COMP = Competition challenge (puzzle and mirror tracing activities).
*p < .05, **p < .01.

Table 4
Fixed effect estimates from the final 2-level multilevel models with child- and maternal-reported depression predicting log transformed cortisol levels in the Trier Social Stress Test.

Log Cortisol (nmol/L)

Child-reported Depression (CDI)				Maternal-reported Depression (CBCL)			
Fixed Effect	γ	*SE*	*t*	Fixed Effect	γ	*SE*	*t*
Intercept	1.224226	0.285814	4.283***	Intercept	1.137038	0.276491	4.112***
Time since waking	-.000012	0.000005	−2.640*	Time since waking	−0.000008	0.000005	−1.691ˆ
Time of last meal	−0.000007	0.000007	−1.067	Time of last meal	−0.000008	0.000006	−1.294
CDI	−0.003945	0.007109	−0.555	CBCL Dep	−0.024665	0.017618	−1.400
Time	0.042049	0.018222	2.308 *	Time	0.038366	0.018939	2.026*
$Time^2$	−0.008602	0.003310	−2.599*	$Time^2$	−0.007429	0.003327	−2.233*
Time*CDI	−0.007030	0.002905	−2.420*	Time*CBCL	−0.001270	0.008555	−0.148
$Time^2$*CDI	0.001141	0.000528	2.162*	$Time^2$*CBCL	0.000353	0.001502	0.235

Log Cortisol (nmol/L)

Child-reported Depression (CDI)				Maternal-reported Depression (CBCL)			
Random Effect	Variance	SD	X^2 (df)	Random Effect	Variance	SD	X^2 (df)
Intercept	0.07049	0.26550	420.92(38)***	Intercept	0.05457	0.23360	359.05 (385)***
Time slope	0.01035	0.10172	153.20 (40)***	Time slope	0.01176	0.10843	178.89 (40)***
$Time^2$	0.00032	0.01798	130.24(40)***	$Time^2$	0.00034	0.01842	143.04 (40)***
Level-1	0.02623	0.16196		Level-1	0.02410	0.15526.	
$\chi^2(4) = 22.42604$, $p < .001$				$\chi^2(4) = 0.86102$, $p > .500$			

Note. CDI = Child Depression Inventory; CBCL Dep = Child Behavior Checklist depression items; Time = Linear time, $Time^2$ = Quadratic time. χ^2 indicates model fit compared to null model.
ˆ $p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$.

separate analyses reported below.

3.4. Predictors of cortisol

3.4.1. Predictors of cortisol in the TSST-C

The first model assessed the effect of child self-reported depressive symptoms (CDI) and time (centered at baseline) on log-cortisol. Model coefficients and standard errors of fixed and random effects are shown in Table 4. The final model fit the data significantly better than the null model (Table 4). Time since waking and time of last meal were retained as covariates in the final model. The fixed effects for linear and quadratic time were significant, indicating that cortisol increased over time and that the rate of change of cortisol decreased over time, respectively. The random effects (slopes) for linear and quadratic time (i.e., whether there was significant variation in cortisol trajectories and rate of change across individuals) were significant and significantly improved model fit.

The CDI x linear time and CDI x quadratic time interactions were also significant (Table 4), indicating that cortisol trajectories differed as a function of child-reported depressive symptoms. As shown in Fig. 2, individuals with high CDI had declining cortisol across time, whereas individuals with low CDI had increasing cortisol across time. In addition, the CDI x quadratic time interaction suggests that cortisol levels for children with high CDI declined faster than was the case for children with low CDI (Fig. 2). Both the CDI x time (b = -.003, SE = .001, p = .023) and CDI x quadratic time interactions were significant when the model intercept was centered at peak (b = .001, SE = .001, p = .037).

A similar model was conducted to assess the impact of maternal-reported depressive symptoms (CBCL) on cortisol in the TSST. For consistency, time since waking and time of last meal were retained as covariates in the final model. As shown in Table 4, the CBCL x time interactions were not significant but were retained in the final model to facilitate comparison with the model using CDI as a predictor (described above, Table 4). There was also not a significant effect of mother-reported CBCL depressive symptoms in the final model. However, the effect of CBCL depression on cortisol was significant prior to the inclusion of covariates (b = -.033, SE = .016, p = .048) and was possibly washed out due to the limited power of the model with covariates and non-significant interaction terms.

3.4.2. Predictors of cortisol in the competition challenge

In the competition challenge, the fixed effects for linear time (b = -.030, SE = .009, p = .002) and quadratic time (b = .006, SE = .002,

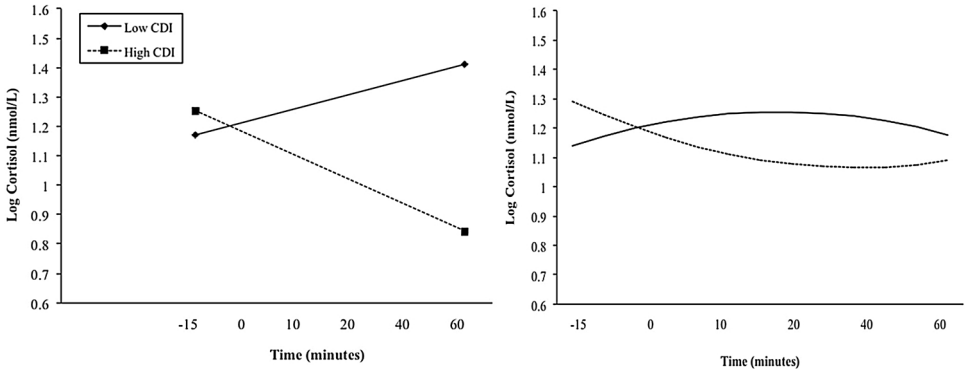

Fig. 2. Interaction between self-reported child depressive symptoms and saliva sampling time predicting log transformed cortisol levels in the Trier Social Stress Test, with time centered at baseline (0 min). The graph on the left models linear time, whereas the graph on the right models quadratic time. Actual collection time was used in all models; however, average collection time is noted on the X axis. Continuous levels of child depressive symptoms were used in analyses, categories of low (-1 SD) and high (+1 SD) depression are only used for graphing purposes.

Table 5
Fixed effect estimates from the final 2-level multilevel models with child- and maternal-reported depression predicting log transformed salivary alpha-amylase in the Trier Social Stress Test.

Log sAA (U/mL)							
Child-reported Depression (CDI)				Maternal-reported Depression (CBCL)			
Fixed Effect	γ	*SE*	*t*	Fixed Effect	γ	*SE*	*t*
Intercept	0.405201	0.854553	0.474	Intercept	0.232750	0.735170	0.317
Time of last meal	0.000003	0.000009	0.357	Time of last meal	0.000003	0.000008	0.320
Age	0.136605	0.070747	1.931ˆ	Age	0.157036	0.061596	2.549*
Ethnicity	0.003226	0.017942	0.180	Ethnicity	0.018852	0.019299	0.977
CDI	0.007400	0.007756	0.954	CBCL Dep	0.018852	0.019299	2.539*
Time	−0.003671	0.009509	−0.386	Time	−0.004504	0.009441	−0.477
$Time^2$	−0.010195	0.007299	−1.397	$Time^2$	−0.009151	0.007269	−1.259
Time*CDI	−0.000327	0.001472	−0.222	Time*CBCL	−0.006634	0.004094	−1.620
$Time^2$*CDI	−0.000542	0.001155	−0.469	$Time^2$*CBCL	−0.000803	0.003228	−0.249

Log sAA (U/mL)							
Child-reported Depression (CDI)				Maternal-reported Depression (CBCL)			
Random Effect	Variance	SD	X^2 (df)	Random Effect	Variance	SD	X^2 (df)
Intercept	0.06703	0.25890	555.69 (31)***	Intercept	.06	.24	536.63 (33)***
$\chi^2(1) = 93.31703, p < .001$				$\chi^2(1) = 83.03000, p < .001$			

Note. CDI = Child Depression Inventory; CBCL Dep = Child Behavior Checklist depression items; Time = Linear time, $Time^2$ = Quadratic time. χ^2 indicates model fit compared to null model.
ˆ $p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$.

$p = .001$) were significant, indicating that cortisol decreased over time and that the rate of change of cortisol increased over time, respectively, in the competition challenge. However, CDI and CBCL effects, as well as all interactions were not significant.

3.5. Predictors of sAA

3.5.1. Predictors of sAA in the TSST-C

Separate models were run to assess the trajectories of sAA. Child age, ethnicity, and time of last meal were retained as significant covariates in the final model. There were no significant fixed or random effects of time or quadratic time, and child-reported CDI and CDI*time interactions did not significantly predict sAA in the TSST (Table 5). In contrast, there was a significant first-order effect of maternal-reported depression on sAA at baseline (0 min) in the TSST, despite the non-significant main effects and interactive effects with time (Table 5). This effect remained significant with the intercept centered at peak ($b = .048$, $SE = .019$, $p = .018$).

3.5.2. Predictors of sAA in the competition challenge

Similar to the TSST, there were no significant effects of linear time or quadratic time on sAA in the competition challenge (Table 6). In addition, the first-order CDI effect as well as all interactions were not significant. In contrast, there was a significant first-order effect of maternal CBCL depression (Table 6), indicating that children with higher maternal reported depression have higher sAA at baseline (0 min) in the competition challenge. This effect was not significant with the intercept centered at peak ($b = .020$, $SE = .017$, $p = .241$).

3.6. Cortisol-sAA coordination

3.6.1. Coordination in the TSST-C

As in the other models with cortisol as the outcome, time since waking and time of last meal were retained as covariates in the final model. First, sAA effects were tested along with child-reported depression. The CDI x sAA, sAA x time, and CDI x sAA x time interactions were all not significant. However, the sAA effect ($b = .240$, $SE = .100$, $p = .017$) was significant, indicating that sAA was positively associated with cortisol at baseline. Similarly, when tested with maternal-reported CBCL depression in the model, although the interaction effects were not significant, the sAA effect ($b = .216$, $SE = .100$, $p = .035$) was significant.

3.6.2. Coordination in the competition challenge

In the competition challenge, the interaction between sAA and CDI was significant (Table 7). As shown in Fig. 3, individuals with higher CDI scores and higher sAA levels had the highest cortisol levels, compared to individuals with lower CDI scores and lower sAA levels. A similar coordination model was tested with maternal CBCL as a predictor; however, all possible interactions with sAA, CBCL, and time were not significant (Table 7).

4. Discussion

The present study assessed cortisol and sAA patterns, as well as cortisol-sAA coordination, in the context of child depressive symptoms while accounting for two primary factors that typically influence these associations: 1) the differential effectiveness of stressor paradigms to elicit cortisol and sAA, and 2) the reporter of depressive symptoms. Findings support the hypothesis that child depressive symptoms are associated with cortisol and sAA activity, as well as cortisol-sAA coordination. However, findings differed based on the challenge and the reporter of depressive symptoms.

Overall, mean cortisol and sAA levels across the entire sample indicate that cortisol increased over time but there was not a significant effect of time on sAA in the TSST, whereas cortisol declined over time but there was not a significant effect of time on sAA in the competition challenge. Consistent with these trajectories, prior studies show that the TSST elicits strong cortisol responses (Buske-Kirschbaum et al., 1997; Gunnar, Talge et al., 2009, 2009b), but elicits a less-pronounced (and non-significant) sAA response in children (e.g., Granger et al., 2006; Stroud et al., 2009). In contrast, we had hypothesized significant increase in sAA levels in response to the effort-based, controllable challenge, but this did not materialize.

Table 6
Fixed effect estimates from the final 2-level multilevel models with child- and maternal-reported depression predicting log transformed salivary alpha-amylase in the Competition challenge.

Log sAA (U/mL)							
Child-reported Depression (CDI)				Maternal-reported Depression (CBCL)			
Fixed Effect	γ	*SE*	*t*	Fixed Effect	γ	*SE*	*t*
Intercept	1.886594	0.548894	3.437**	Intercept	1.785519	0.510878	3.495**
Time of last meal	−0.000020	0.000007	−3.059**	Time of last meal	−0.000026	0.000007	−3.850**
Age	0.079075	0.054933	1.439	Age	0.119203	0.052082	2.289*
Ethnicity	0.038078	0.016392	2.323*	Ethnicity	0.047528	0.016112	2.950**
CDI	0.007999	0.006451	1.240	CBCL Dep	0.049138	0.020500	2.397*
Time	−0.018821	0.009834	−1.914ˆ	Time	0.018152	0.010139	−1.790ˆ
$Time^2$	0.002861	0.005807	0.493	$Time^2$	0.003093	0.005770	0.536
Time*CDI	−0.002264	0.001544	−1.467	Time*CBCL	−0.001691	0.004917	−0.344
$Time^2$*CDI	−0.000344	0.000932	−0.370	$Time^2$*CBCL	−0.002818	0.002835	−0.994

Log sAA (U/mL)							
Child-reported Depression (CDI)				Maternal-reported Depression (CBCL)			
Random Effect	Variance	SD	X^2 (df)	Random Effect	Variance	SD	X^2 (df)
Intercept	0.04897	0.22129	597.41 (29)***	Intercept	0.04394	0.20963	544.00 (29)***
Time slope	0.00137	0.03703	58.14 (32)**	Time slope	0.00160	0.03997	62.74 (32)***
Level-1	0.01115	0.10560		Level-1	0.01105	0.10512	
$\chi^2(1) = 56.55246$, $p < .001$				$\chi^2(1) = 47.93822$, $p < .001$			

Note. CDI = Child Depression Inventory; CBCL Dep = Child Behavior Checklist depression items; Time = Linear time, $Time^2$ = Quadratic time. χ^2 indicates model fit compared to null model.
ˆ $p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$.

The design of this study, particularly the use of the TSST and competition challenge, was based on prior theory and research on the types of challenges that should activate the HPA axis and ANS, respectively. It was expected that the TSST, a social-evaluative and uncontrollable task, would elicit strong cortisol reactivity and given prior work (Granger et al., 2006; Stroud et al., 2009), it is not surprising that the TSST did not evoke a strong sAA response. The failure of the competition challenge to evoke sAA might be explained by the ambiguity regarding which kinds of challenges elicit sAA reactivity. Early theory suggested that controllable and effortful challenges might best elicit ANS responsivity (Frankenhaeuser et al., 1980; Lundberg & Frankenhaeuser, 1980). Yet, the research thus far provides conflicting evidence for what kind of tasks elicit ANS activity, with some work showing that controllable, effort-based, challenges elicit ANS activity

Table 7
Fixed effect estimates from the final 2-level multilevel models with child- and maternal-reported depression interacting with salivary alpha-amylase (i.e., coordination) predicting salivary cortisol in the Competition challenge.

Log Cortisol (nmol/L)							
Child-reported Depression (CDI)				Maternal-reported Depression (CBCL)			
Fixed Effect	γ	*SE*	*t*	Fixed Effect	γ	*SE*	*t*
Intercept	0.837011	0.213569	3.919***	Intercept	0.729816	0.182952	3.989**
Time since waking	−0.000003	0.000005	−0.620	Time since waking	−0.000002	0.000005	−0.494
Time of last meal	−0.000007	0.000005	−1.351	Time of last meal	−0.000005	0.000005	−1.173
CDI	0.000926	0.004692	0.197	CBCL Dep	−0.014164	0.011327	−1.250
Time	−0.028540	0.009768	−2.922**	Time	−0.036236	0.010071	−3.598**
$Time^2$	0.006327	0.001806	3.504**	$Time^2$	0.008173	0.001867	4.377***
Time*CDI	0.000571	0.001638	0.348	Time*CBCL	−0.000576	0.003776	−0.153
$Time^2$*CDI	−0.000449	0.000306	−1.469	$Time^2$*CBCL	−0.000170	0.000656	−0.259
sAA	0.037310	0.118262	0.315	sAA	−0.196210	0.129961	−1.510
sAA*CDI	0.059050	0.019902	2.967**	sAA*CBCL	0.052060	0.035907	1.450

Log Cortisol (nmol/L)							
Child-reported Depression (CDI)				Maternal-reported Depression (CBCL)			
Random Effect	Variance	SD	X^2 (df)	Random Effect	Variance	SD	X^2 (df)
Intercept	0.02492	0.15785	173.88 (39)***	Intercept	0.02488	0.15774	169.27 (38)***
Time slope	0.00049	0.02216	72.63 (41)**	Time slope	0.00046	0.02140	67.67 (40)***
Level-1	0.02201	0.14836		Level-1	0.02224	0.14914	
$\chi^2(1) = 92.54454$, $p < .001$				$\chi^2(1) = 85.45759$, $p < .001$			

Note. CDI = Child Depression Inventory; CBCL Dep = Child Behavior Checklist depression items; Time = Linear time, $Time^2$ = Quadratic time. χ^2 indicates model fit compared to null model.
ˆ $p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$.

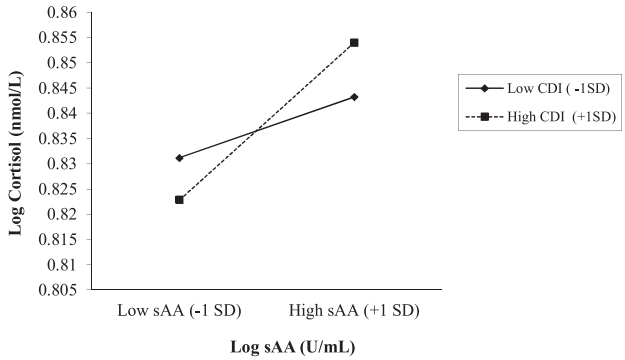

Fig. 3. Interaction between self-reported child depressive symptoms and log transformed salivary alpha amylase (sAA) predicting log-transformed cortisol levels in the Competition challenge. Continuous levels of child depressive symptoms and sAA were used in analyses, categories of low (−1 SD) and high (+1 SD) are only used for graphing purposes.

(e.g., Chatterton et al., 1996; Laurent et al., 2012), and other work showing that physical challenges elicit strong ANS activation (Skoluda et al., 2015). More research is needed to understand the elements of challenges that elicit sAA. Related, it is also possible that the competition challenge used here is simply not a strong enough stressor to elicit HPA or ANS activation. In addition, perhaps alternative approaches to assessing ANS activation, including real-time measures (e.g., respiratory sinus arrhythmia, skin conductance), would yield different findings, given that sAA reactivity is fleeting and it may be difficult to capture peak sAA. In addition, given that children, relative to adolescents, show less pronounced cortisol and sAA reactivity (e.g., Granger et al., 2006; Stroud et al., 2009), it is also possible that a combination of more robust and targeted stressors are needed to elicit cortisol and sAA at this age. As the first study to assess child cortisol and sAA in two distinct challenges, this research provides a starting point and indicates that additional work is needed to uncover exactly what kinds of challenges elicit strong sAA reactivity.

4.1. Associations between child depressive symptoms, cortisol, and sAA reactivity

4.1.1. Child depression symptoms and cortisol

In addition, we found interesting effects of child- and maternal-reported depression on cortisol. A typical cortisol response to a stressor includes an increase in cortisol from baseline levels after the onset of a stressor (reactivity) followed by a decline or return to baseline (recovery). In the TSST, children with higher depressive symptoms had higher cortisol levels at initial baseline (- 15 min) compared to children with lower depression scores (see Fig. 2). Further, children with higher self-reported depressive symptoms failed to mount an appropriate cortisol response to the TSST, such that their cortisol trajectories declined across time, compared to those with lower depression scores. Together, this indicates that although children with higher depressive symptoms have greater baseline cortisol levels in anticipation of the stressor, their cortisol declines across time in response to a social-evaluative challenge. The present findings, in combination with prior work, suggest that depressive symptoms are associated with lower/declining cortisol reactivity in community and clinical samples, both during adolescence and childhood. Given that there is a marked increase in depression after the onset of puberty (Patton et al., 2008; Stroud, Papandonatos, Williamson, & Dahl, 2011), these findings indicate that declining cortisol trajectories and higher anticipatory cortisol levels are associated with depressive symptoms earlier in childhood (prior to puberty). Identifying early neuroendocrine markers for childhood depression has important clinical and research implications. Ultimately, longitudinal research is needed to determine if declining cortisol trajectories in response to the TSST in childhood is predictive of chronicity and severity of depressive symptoms in adolescence.

4.1.2. Cortisol responsivity in two different challenges

Number of child-reported depressive symptoms was inversely associated with cortisol reactivity in the TSST, but not associated with cortisol in the competition challenge. In addition, higher depressive symptoms predicted greater baseline cortisol levels in TSST, but the competition challenge. Thus, depressive symptoms might impact anticipatory cortisol levels differentially based on the type of forthcoming challenge. It is possible that we did not find a statistical difference in cortisol trajectories between those with high/low depressive symptoms in the competition challenge because cortisol did not significantly change in the competition challenge for all children, regardless of depression level. Collectively, these results might reflect that children with higher depressive symptoms demonstrate lower cortisol reactivity in both challenges. These are intriguing findings that suggest that cortisol reactivity should be assessed in distinct challenges.

Using an adolescent sample, Laurent et al. (2016) examined the association between internalizing problems and cortisol reactivity in two distinct challenges. Laurent et al. (2016) found that broader maternal-reported internalizing symptoms predicted an earlier cortisol peak in the interpersonal rejection challenge. In contrast to the present study findings, internalizing behaviors were not associated with cortisol reactivity in a TSST-like performance challenge. In sum, the findings of the current study, along with prior research, suggest that associations between internalizing behavior and cortisol reactivity differ depending on the nature of the challenge.

4.1.3. Child depressive symptoms and sAA

This is one of the first studies to show that maternal-reported depressive symptoms are associated with higher sAA levels at baseline (0 min) in the TSST and in the competition challenge. This finding is particularly noteworthy given that sAA did not significantly change over time in the TSST or competition challenge (Tables 5 and 6), yet individual variability in maternal-reported depressive symptoms predicted greater baseline sAA in these challenges. This highlights the importance of assessing child depressive symptoms, using both parent and child ratings, to predict sAA levels, even during childhood, a time of relative physiological hypo-responsivity.

4.2. Coordination of cortisol and sAA

We found significant effects of sAA on cortisol in both the competition challenge and TSST, indicating that, at baseline (0 min), higher sAA predicted higher cortisol in both challenges. The association between sAA and cortisol signifies the presence of cross-system coordination and highlights the importance of measuring sAA in conjunction with cortisol. In addition, we found that children with higher self-reported depressive symptoms who experienced higher sAA also exhibited higher cortisol in the competition challenge (Fig. 3). Thus, in the competition challenge, child-reported depressive symptoms are associated with high activation in both HPA and ANS systems. This is interesting, given that child depressive symptoms alone, without sAA in the model, did not predict cortisol levels in the competition challenge. Here coordination is based on baseline levels (centered at 0 min) of cortisol and sAA, *not* trajectories across time. This finding can be understood in the context of Bauer et al.'s (2002) *additive model,* which suggests that non-optimal functioning occurs when the HPA and ANS systems are synchronously activated at a high level and optimal functioning occurs when each system is activated at a moderate level or when one system is highly activated while the other is not (i.e., asynchronous activity) (Bauer et al., 2002).

It is difficult to compare the present study results to other coordination findings because prior studies use different challenges. Similar to the present results, greater internalizing problems were

associated with synchronously high cortisol and sAA coordination, using a composition of challenges to elicit stress (El-Sheikh et al., 2008). In contrast, the TSST-C produced lower cortisol and lower sAA levels in those with greater internalizing problems (Bae et al., 2015). Although we did not find coordination in the TSST-C, the coordination results for the competition challenge can be understood in the context of this prior research. In anticipation of the competition challenge, children with higher self-reported depressive symptoms evinced *additive* coordination (i.e., high cortisol and high sAA). Given that the competition challenge did not evoke an increase in cortisol or sAA for the entire sample, this cross-system amplification (high cortisol/high sAA) is possibly an atypical response. Similarly, Bae et al. used the potent TSST-C challenge to elicit coordination at the low level (i.e., low cortisol and low sAA for children with higher internalizing behavior problems), a pattern of coordination that was similarly interpreted as atypical, given the nature of the challenge. All in all, the present findings, which differ across challenges, underscore the need to examine depressive symptoms in relation to cortisol-sAA coordination across variant stressors.

4.3. The reporter of depressive symptoms impacts associations with cortisol and sAA responsivity

This study was designed to control for different raters of depressive symptoms, given that parent and child reports of psychopathology often do not align (Achenbach & Rescorla, 2001; De Los Reyes & Kazdin, 2005). In the current study, child-reported depressive symptoms were not significantly correlated with maternal-reported symptoms ($r = .18$, $p = .25$). Given the low correlation, it is not surprising that findings differed by reporter. Child-reported symptoms predicted cortisol and sAA-cortisol coordination, whereas maternal-reported symptoms only predicted sAA. To our knowledge, this is the first study to assess multiple reporters of depressive symptoms in relation to cortisol and sAA in multiple challenges. Given the limited research on sAA, it is difficult to know why maternal, but not child-reported difficulties predicted sAA. It is possible that parents are attuned to specific aspects of depressive problems that are more strongly related to the ANS. However, this is speculative, and future work is needed to further explore child and parent reported difficulties in relation to sAA.

4.4. Study strengths

To date, research examining the association between cortisol reactivity and childhood depressive symptoms has been complicated by several methodological limitations, including single stressor designs, absence of the examination of physiological coordination, and different raters of depressive symptoms. The current study aimed to address these issues. First, this study measured cortisol and sAA reactivity within two different challenges. The distinct findings are consistent with prior work showing unique physiological reactivity to particular challenges (e.g., Laurent et al., 2016). Second, this study examined the coordination of cortisol and sAA reactivity. Third, this study used both mother and child reports of depressive symptoms, which proved fruitful, as some findings differed based on the reporter of symptoms, similar to prior work (e.g., Chen et al., 2015).

4.5. Limitations and future directions

This study has limitations that should be addressed in future work. First and foremost, we used a small sample to test complex multilevel models. It is possible that the nonsignificant findings are the product of limited power. Although this study has the strength of simultaneously investigating several important factors in a within-subject design, findings should be replicated with a larger sample to determine reliability and generalizability. Related to generalizability, this study used a community sample of children who were relatively low risk in terms of their socio-demographic profile. Replication with more at-risk and clinical samples is necessary to generalize findings. Lastly, this study used a novel competition challenge, designed in an attempt to evoke strong sAA reactivity. Future research is needed to determine the validity of this competition challenge to evoke HPA or ANS activity (e.g., sAA or real-time markers) in diverse samples.

5. Conclusion

Taken together, the results of this study indicate that child depressive symptoms are associated with cortisol and sAA responses, as well as sAA-cortisol coordination, but these effects varied by the challenge used to elicit stress and by the reporter of depressive symptoms. These findings point to the complexity of the association between child depressive symptoms and stress physiology, indicating that future work is needed to decipher precisely how stress physiology is associated with depressive symptoms in childhood.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopsycho.2019.107808.

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