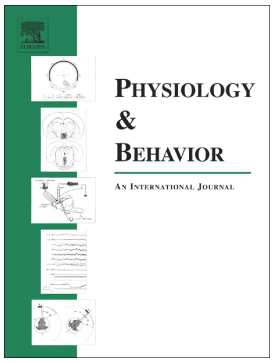

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Sex differences in biological response to peer rejection and performance challenge across development: A pilot study

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Abstract

A pilot study of sex differences in biological response to peer rejection and performance challenges across development was conducted. Participants were 59 typically-developing children (ages 8-17; 58% girls); 59 children completed one challenge: 37 completed both challenges. Following a habituation session, participants completed peer rejection (exclusion challenges) and/or performance (speech, arithmetic, tracing) stress sessions. Saliva cortisol and alpha amylase (AA) were measured throughout. Post-pubertal girls showed increased AA and equivalent cortisol output in response to rejection vs. performance; pre-pubertal girls showed heightened cortisol and AA response to performance vs. rejection. Boys showed similar biological responses across puberty, with pre- and post-pubertal boys demonstrating heightened cortisol, but equivalent AA output in response to performance vs. rejection stressors. Although results are preliminary, they suggest increases in relative sensitivity to rejection vs. performance stressors and malleability of stress response across development in girls, but stability of stress response across development in boys. Future, larger-scale, longitudinal studies are needed.

Keywords: Sex differences, development, stress, cortisol, autonomic, peer rejection, performance, puberty

1. Introduction

The transition from middle childhood to adolescence represents a time of tremendous, and potentially tumultuous change, including changes in social and academic environments and expectations, superimposed on the neuroendocrine and morphological changes of puberty [1-4]. Adolescence is also a critical time for the emergence of sex differences in numerous aspects of functioning (social, academic, emotional, and physical), and for the initiation of trajectories leading to later adult roles for girls and boys [5-8]. A growing body of literature has also highlighted the adolescent transition as a critical period of brain plasticity and sensitivity to environmental stimuli. In particular, it is a key period of maturation of brain circuits regulating social and affective processing [9-13] as well as brain circuits and structures mediating the stress response [14-16]. However, although a rapidly advancing body of theory and empirical research has examined stress response regulation in adults and in infancy/childhood, much less is known regarding stress response regulation over the adolescent/pubertal transitions [17-19]. Furthermore, there is a remarkable paucity of studies of sex differences in stress response over the adolescent transition despite potential implications for understanding sex differences in trajectories of emotional, social, and academic functioning.

Biological stress response systems are necessary for survival and have been conserved across species, suggesting a fundamental evolutionary adaptation [20-22]. Links between biological stress response and emotional, physical and social functioning as well as learning have also been shown [23, 24]. The peripheral neuroendocrine stress response involves two major systems: the hypothalamic pituitary adrenocortical (HPA) axis and the autonomic nervous system (ANS). Activation of the HPA axis results from a cascade of neural events culminating in the release of glucocorticoids (primarily cortisol in humans) from the adrenal cortex over

minutes, hours, and days. The ANS controls the more immediate response to stress and consists of two coordinated systems: sympathetic (controls activating processes) and parasympathetic (controls vegetative processes), whose integration determines level of arousal. Saliva α-amylase (AA), an enzyme produced in the oral mucosa via beta and alpha adrenergic mechanisms, has been identified as a non-invasive surrogate marker of ANS activity [25-27].

Preclinical studies have demonstrated increased HPA and ANS stress response over the pubertal transition [28-31]. Cross-sectional and longitudinal studies of basal cortisol levels in humans have also shown increased cortisol in late-pubertal and older adolescents [32-34]. Complementing preclinical studies and studies of basal cortisol, an emerging line of human research, including three cross-sectional and one longitudinal study have documented a fundamental shift in HPA and ANS stress response over the adolescent/pubertal transition [35-38]. Synthesizing across studies, older and post-pubertal adolescents showed increased cortisol and AA response to stress versus younger or pre-pubertal children. One longitudinal study — a follow up of Sumter et al. [36] — confirmed the fundamental shift in HPA and ANS reactivity in a 2-year, within-participants design over the adolescent/pubertal transition [38]. van den Bos et al. [38] demonstrated both between and within-participants increases in cortisol and AA response to the Leiden Public Speaking Task (LPST) over the 2-year period, with the influences of puberty outweighing those of age.

Preclinical studies have also shown consistent sex differences in HPA stress systems in adults and across the pubertal/adolescent transition. In rodent models, adult females have shown consistently higher basal hormone levels, greater and more rapid responses to acute stressors, and decreased adaptation to chronic or repeated stressors relative to adult males [39]. There is also evidence from rodent models that heightened HPA response in females also emerges over

puberty [40]. In adult humans, males have shown relatively consistent increased HPA and ANS reactivity to most laboratory psychological stressors (performance and public speaking tasks) versus females [41, 42], with evidence for high magnitude differences during both reactivity to and recovery from stressors [43]. However, studies of sex differences in HPA and ANS stress systems across development in humans have been inconsistent. Although girls have shown higher *basal* levels of cortisol than boys post-puberty [44-47], studies of sex differences in *reactivity* across the adolescent transition have revealed increased response in post-pubertal boys, increased response in post-pubertal girls, and no sex differences [35, 36, 38, 44, 48, 49]. In their longitudinal study, van den Bos et al. [38] found increased cortisol and AA response to the LPST in girls versus boys; however, sex differences were no longer evident after control for pubertal development. Studies of sex differences in the ANS are also equivocal, with inconsistent results for studies of heart rate and sympathetic and parasympathetic tone, but increased blood pressure response to stress in adolescent boys [50].

In sum, while evidence is relatively consistent for increases in HPA and ANS stress response over the pubertal transition, the direction of sex differences in HPA and ANS stress response over the pubertal transition in humans is unclear. Emerging and converging models from psychology, psychiatry, and neuroscience have highlighted sex differences in greater communal orientation in females versus greater agentic orientation in males; increased exposure to affiliative/interpersonal stressors in girls and to agentic/ achievement stressors in boys; increased sensitivity to affiliative/interpersonal stressors in girls and women; and more robust associations between affiliative stressors and depression in girls and women, but agentic stressors in boys and men [51-55]. We previously proposed [56] that the differential salience of interpersonal and performance stressors for women versus men might help to elucidate

discrepancies between preclinical and human studies of sex differences in stress response in adults (i.e. greater female reactivity in preclinical studies, but greater male reactivity in human studies). Specifically, our group and others have proposed that peer rejection is a key interpersonal construct of relevance for understanding sex differences in stress response and depression [56-58]. In support of this hypothesis, we showed increased cortisol response to peer rejection stressors in women, but increased cortisol response to achievement challenges in men in a healthy adult sample [56]. Furthermore, in a study of typically developing children and adolescents, we found increased cortisol and AA response to both performance and peer rejection challenges in adolescents (13+ years) versus children (<12 years) [37]; however, sex differences were not examined, and all participants completed only one type of stressor.

The present study is a pilot study of sex differences in HPA (cortisol), and ANS (AA) response to peer rejection and performance stressors in a typically developing sample of 59 children. The present study extends our prior work through investigation of sex differences, and inclusion of a subset of participants who completed both peer rejection and performance challenges. We explored the hypothesis that girls would show altered biological sensitivity to stressor domain over the pubertal transition, with increased response to peer rejection versus performance stressors in post-pubertal girls, and increased response to performance versus peer rejection stressors in pre-pubertal girls and older and post-pubertal boys. We also explored cross-stressor (performance vs. peer rejection) consistency in HPA and ANS response in the subset of participants who completed both peer rejection and performance stressors.

2. Material and Methods

2.1. Participants

Participants were 59 healthy children and adolescents ages 8 to 17 (58% girls; *M*= 12.6,

SD=2.6) recruited through community and online postings, including 28 children (8-12 years, 57% girls) and 31 adolescents (ages 13-17 years, 58% girls). According to Tanner pubertal stage criteria, 44% of the sample was in the early to middle stages of puberty (I-III) and 56% of the sample was in the later stages of puberty (IV, V). Exclusion criteria were based on factors known to influence cortisol and alpha amylase (AA) reactivity, including use of oral contraceptives, thyroid medications, steroids, and psychotropic medications [59-61]. Also excluded were participants with a diagnosis of autism or mental retardation, a history of psychological or behavioral problems, or current physical illness. All participants denied smoking and regular alcohol or drug use.

All 59 participants completed one stress session; a subset of 37 participants completed two stress sessions. Specifically, in the initial phase of this pilot study, 16 participants were offered one stress session only (n=13 performance sessions; n=3 peer rejection). In the second stage of this study, 43 participants were asked to complete two stress sessions. Six did not continue the study after the first stress session (n=4 performance; n=2 peer rejection), leaving 37 participants who completed both stress sessions. Rates of continuation after the first stress session were 86% overall—87% (27 out of 31 participants continuing) when the performance session was run first, and 84% (10 out of 12 participants continuing) when the peer rejection session was run first (10/12).

2.2. Procedures

Protocols and procedures were reviewed and approved by a Lifespan Hospitals Institutional Review Board. Informed consent was obtained from parents; assent was obtained from children and adolescents. The study included two or three sessions conducted on separate days, with each lasting approximately 2 hours. All sessions began between 14:00 and 17:00 to

control for circadian cortisol variation. The first session was a "rest" session, in which participants habituated to the laboratory and saliva sampling in order to attenuate the influence of laboratory novelty on neuroendocrine responses prior to the stress induction sessions. During the "rest" session, participants watched G-rated movies and television shows and completed questionnaires. The second and third sessions were performance and/or peer rejection stress induction sessions administered in random sequence and based on scheduling constraints for peer rejection sessions. Twenty-seven participants completed the performance session first; ten participants completed the peer rejection session first.

Both stress sessions included a 30-minute baseline period in which participants watched G-rated movies and television shows, three stressors, lasting 10, 5, and 5 minutes, respectively, and a 60-minute recovery period in which participants completed questionnaires and watched G-rated movies and television shows. Tasks for the performance session included: a speech (5 minutes preparation, 5-minute speech), mental arithmetic (5 minutes), and mirror tracing (5 minutes). Tasks for the peer rejection session were three exclusion challenges (10, 5, and 5 minutes) with gender/age-matched confederates. Nine saliva samples were collected over the baseline, stressor, and recovery periods to optimize timing for assessment of cortisol and AA response to the stress tasks (See Measures below). Timing of saliva samples over the course of the performance and peer rejection sessions is shown in Figure 1. Participants were asked to refrain from food and drink (besides water), exercise, and caffeine at least 1 hour prior to the stress session. Extensive debriefing included: (a) debriefing with child/adolescent participant, (b) debriefing with parent and child/adolescent participant, and (c) positive interaction with confederates/audience. Participants and parents were then compensated for their time; child participants received $45 and parent participants received $40 for each session.

Insert Figure 1 about here

2.3. Stressors

2.3.1. Performance Challenges. Performance-oriented tasks were based on an adaptation of the Trier Social Stress Test for Children (TSST-C) [62] described previously [37]. Tasks included: a) public speaking, in which participants were given 5 minutes to prepare, then were asked to speak on academic topics (e.g., English, Science, History) for 5 minutes, with difficulty adjusted based on participant age; b) mental arithmetic, involving 5 minutes of serial subtraction under time pressure, with difficulty adjusted based on participant age and performance; c) mirror star tracing, adapted from Allen and Matthews and involving 5 minutes of tracing the figure of a six-sided star while viewing only its mirror image using a mirror star tracing apparatus (Layfayette Instruments, 1987) with errors counted and marked by sound and light. All tasks were performed before a two-member audience who remained stern and wrote "notes" on a clipboard during the procedures.

2.3.2. Peer rejection challenges. These challenges involve three peer rejection interactions based on an adaptation for children and adolescents of the Yale Interpersonal Stressor (YIPS; described previously [37]). The YIPS-Child Version (YIPS-C) involved interactions with two trained, same-sex, similar-age confederates who subtly excluded the participant by bonding with each other, leaving the participant out of their conversations, and having different interests and activities than the participant. Participants were told that we were studying "how kids get to know one another" and that they were to discuss specified topics, while "getting to know one another." Exclusion interactions focused on three topics: weekend activities, family, and friends, discussed in 10-, 5-, and 5-minute segments, respectively. The

initial 10-minute segment included 5 minutes of introductions and 5 minutes of discussion of weekend activities. Confederates used a variety of verbal and nonverbal techniques to exclude the participant, while connecting well with each other, with exclusion building gradually over the course of the three interaction segments.

2.3.3. Stressor validation: Task Perception Questionnaire (TPQ). TPQ's were administered to validate conceptualization of the performance versus social nature of the two stress sessions. Questions included-forced choice formats and Likert (3-point) scales regarding each of the three tasks within the performance and peer rejection sessions. When asked to choose, 90-95% of participants who completed the peer rejection interaction tasks rated them as more "like talking to people" than "like performing something" or "like school or homework"; while 72-98% participants who completed the performance interaction tasks rated them as more "like performing something" or "like school or homework" than "like talking to people" (all p's< .001). In the Likert scale questions, higher ratings were assigned to the performance task than the peer rejection task on "times you had to perform," "you were trying to reach a goal," "school or homework," and "doing badly in school" (t's>2.9, p's≤.01), while the peer rejection task received higher ratings on "meeting new people," "activities you do with other kids," "hanging out," and "times you have felt left out" than the performance task (t's>2.3, p's≤.03). Results support the construct validity of the peer rejection and performance stressors.

2.4. Measures

2.4.1. Saliva Cortisol and Alpha Amylase (AA). Nine whole saliva samples were collected from each participant by passive drool over the course of each stress session [63]. See Figure 1 for timing of the 9 saliva samples. Following collection, samples were frozen at –80 degrees Celsius until shipment on dry ice to the laboratory of Clemens Kirschbaum, Ph.D.

(Dresden University), where cortisol and AA assays were conducted. *Saliva cortisol* is a reliable and valid measure of free cortisol levels [64, 65] useful for assessing acute HPA changes due to stress and in studies of children and adolescents. Saliva free cortisol concentrations were measured using a commercially-available chemiluminescence-immuno-assay (CLIA) with high sensitivity (0.16 ng/ml; IBL, Hamburg, Germany). Intra and inter-assay coefficients of variation (CV) for cortisol were below 8%. *AA* is an enzyme produced in the oral mucosa via beta and alpha adrenergic mechanisms, that is proposed as a surrogate marker for ANS activity [25-27]. Concentration of AA was measured using an enzyme kinetic method reaction assay that employs a substrate reagent (a-amylase EPS Sys: Roche Diagnostics, Mannheim, Germany). The enzymatic action of AA on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm using a standard ELISA reader (Anthos Labtech HT2, Anthos, Krefeld, Germany). The amount of AA activity present in the sample is directly proportional to the increase (over a 2-minute period) in absorbance at 405 nm. Intra and inter-assay CVs were less than 10 and 12%, respectively.

2.4.2. Pubertal Development. Tanner pictures were utilized as a proxy for pubertal development (Marshall & Tanner, 1969, 1970). Tanner pictures depicted different stages of each Tanner criterion (breast (B) and pubic hair (PH) for girls; genital (G) and PH for boys); ratings for the Tanner pictures were obtained in the context of an adapted version of the picture-based interview about puberty [66, 67]. Tanner scores in the present study were based on the mean of Tanner B/G and PH from the Tanner pictures. Due to small numbers of participants in each Tanner stage and gender in this n=59 sample, Tanner stages were subsequently dichotomized into at early-mid or pre-pubertal (I-III) vs. late (IV-V) or post-pubertal groups.

2.5. Data Analyses

2.5.1. Preliminary calculations. Cortisol and AA response to performance and peer rejection stressors were summarized utilizing area under the curve with respect to ground (AUCg) calculated using the trapezoidal rule [68, 69] for nine cortisol and AA time points. The first saliva sample was utilized as the baseline sample for both cortisol and AA; baseline cortisol was modeled in the logarithmic scale; baseline AA was modeled in the square root scale. Two pubertal groups included: Pre-pubertal (Tanner I-III) and Post-pubertal (Tanner IV-V) groups.

2.5.2. Main analyses. The study design was a 2 (stressor domain) X 2 (pubertal group) X 2 (sex) factorial in which pubertal group and sex were between-participants factors and stressor domain was a within-participant factor. Each outcome of interest (cortisol, AA) was analyzed using 3-way analyses of covariance (ANCOVAs) with AUC as the dependent variable (DV); stressor domain, pubertal group, and sex as the independent variables (IVs); and baseline value of the outcome as the sole model covariate. Our approach of measuring AUC with respect to ground (AUCg) adjusted for baseline was selected in lieu of AUC with respect to increase (AUC_I) due to difficulty in conceptualizing AUC_I for participants in whom one or more measurements have values lower than the initial baseline [68, 69]. Model estimation utilized generalized least squares with normal errors, heterogeneous variances for each Pubertal group X Stressor domain X Sex combination, and correlated stress sessions at the individual participant level. Session order (order of performance vs. peer rejection sessions) was tested as a covariate in the ANCOVA models, but was not significantly associated with either cortisol or AA AUC adjusted for baseline.

Three-way interactions were elucidated using the methodology of Cohen & Cohen [70] (Table 1). Specifically, “simple effects” of stressor domain, pubertal group and sex were obtained by calculating AUC differences in the logarithmic scale for performance vs. peer

rejection, calculated separately for pre- and post-pubertal girls and boys. Next, Stressor Domain X Pubertal Group interactions were obtained by calculating differences of the log-AUC differences across pubertal groups, still calculated separately for boys and girls. These sex-specific 2-way interaction effects were finally compared to each other to test for the presence of 3-way Stressor Domain X Pubertal Group X Sex interactions. For ease of interpretation, all interaction findings were then back-transformed to the original scale. As a result, differences-of-differences in log-AUCs appear as ratios of AUC ratios in Table 1.

Of note, effects of stressor domain refer to *relative* differences in cortisol and AA output in response to performance vs. peer rejection stressors.

3. Results

3.1. Sample Description

The sample included 58% girls. Average age was 12.6 years, (*SD*=2.6). Race/ethnicity of the sample included 37% minorities (27% Hispanic, 5% African-American, 5% Asian) and 63% Non-Hispanic Caucasian. Average age of the primary parent was 42.4 years, *SD*=6.4. According to Tanner pubertal stage criteria, 44% of the sample was in the early to middle stages of puberty (I-III) and 56% of the sample was in the later stages of puberty (IV, V). Based on Hollingshead five-factor index [71], 25% of the sample was of high SES (Hollingshead=1), 53% were of middle SES (Hollingshead=2 or 3), and 22% were of low SES (Hollingshead score=4 or 5). Seventy-five percent of primary parents were married; 71% of primary parents and 81% of spouse/partners were employed. Of primary parents, 20% had a high school degree or less; 19% did not complete college; 41% had a college degree, and 20% completed a graduate degree. Of available partners 20% had high school degree or less; 19% did not complete college; 41% had a college degree, and 20% completed a graduate degree. Demonstrating the normative nature of

the sample, T-scores on the Child Behavior Checklist (CBCL), Internalizing Problems=48.4 (*SD*=9.6, Med=48) and Externalizing Problems= 46.6 (*SD*=8.9, Med= 47), were well below suggested clinical screening cutoffs (T≥64) [72, 73]. T-scores on the Child Depression Inventory were 43.8 (*SD*=6.4, Med=42) and the Revised Child Manifest Anxiety Inventory were 42.6 (*SD* =9.1, Med=43), well below suggested clinical screening cutoffs (T's ≥ 65 and 60, respectively) [74, 75].

3.2. Influence of pubertal group and sex on cortisol output in response to performance and peer rejection stressors

Overall, 53% and 33% of the sample showed a biologically meaningful (defined as ≥ 2X intra-assay CV and ≥2X assay sensitivity [76]) cortisol increase in response to the performance and peer rejection stressors, respectively. Figure 2a shows mean raw cortisol levels in response to performance and peer rejection stressors in pre and post-pubertal girls and boys. After adjustment for between-group differences in baseline cortisol levels, we found a significant Stressor Domain X Pubertal Group X Sex interaction (p=.008). Decomposition of this 3-way interaction (Table 1 and Figure 3) revealed that relative cortisol output in response to performance vs. peer rejection differed by pubertal group for girls, but not boys. Specifically, model-based estimates revealed that pre-pubertal girls showed 2X greater cortisol output in response to performance vs. peer rejection stressors (AUC Ratio=2.00, 95% CI=1.48-2.70, p<.001), whereas post-pubertal girls showed similar cortisol output in response to performance and rejection stressors (AUC Ratio=.92, 95% CI=.73-1.14, p=.439). This difference between greater cortisol response to performance vs. peer rejection stressors in pre-pubertal girls, but equivalent cortisol response performance and peer rejection stressors in post-pubertal girls, resulted in a significant Stressor Domain X Pubertal Group interaction among girls (post vs. pre

Ratio of AUC Ratios=0.46, 95% CI=0.31-0.67, $p<.001$). In contrast, both pre- and post-pubertal boys showed approximately 50% increased cortisol output in response to performance vs. peer rejection stressors (pre-pubertal AUC Ratio=1.54, 95% CI=1.14-2.09, $p=.007$; post-pubertal AUC Ratio=1.48, 95% CI=1.18-1.85, $p=.001$). Thus, there was no evidence of a Stressor Domain x Pubertal Group interaction among boys (post vs. pre-pubertal Ratio of AUC Ratios=0.96, 95% CI=0.66-1.40, $p=.84$).

Insert Figures 2 and 3 and Table 1 about here

3.3. Influence of pubertal group and sex on alpha amylase (AA) output in response to performance and peer rejection stressors

Overall, 80% and 62% of the sample showed a biologically meaningful (defined as as ≥ 2X intra-assay CV [76]) AA increase in response to performance and peer rejection stressors, respectively. Figure 2b shows mean raw AA levels in response to performance and peer rejection stressors in pre- and post-pubertal girls and boys. Although baseline differences by pubertal group and sex are evident in the raw data (Figure 2b), after adjustment for baseline AA levels, a significant Stressor Domain X Pubertal Group X Sex interaction emerged ($p<.033$). Paralleling our cortisol findings, decomposition of this 3-way interaction (Table 1 and Figure 3) revealed that relative AA output in response to performance vs. rejection differed by pubertal group for girls, but not for boys. Specifically, model-based estimates revealed that pre-pubertal girls showed 36% increased AA output in response to performance vs. peer rejection (AUC Ratio=1.36, 95% CI=1.05-1.76, $p=.02$), whereas post-pubertal girls showed 23% attenuated AA output in response to performance vs. peer rejection (AUC Ratio= 0.77, 95% CI=0.65-0.92, $p=.004$). This resulted in a significant Stressor Domain x Pubertal Group interaction for girls

(post vs. pre Ratio of AUC Ratios=0.57, 95% CI=0.42-0.77, p<.001). In contrast, both pre- and post-pubertal boys showed approximately 20% increased AA output in response to performance vs. peer rejection stressors (pre-pubertal AUC Ratio=1.25, 95% CI=0.91-1.71, p=.142; post-pubertal AUC Ratio=1.16, 95% CI=1.03-1.30, p=.017). Thus, no significant Stressor Domain X Pubertal Group interaction emerged for boys (post vs. pre-pubertal Ratio of AUC Ratios=0.93, 95% CI=0.68-1.27, p=.633).

3.4. Associations of cortisol and AA measures across performance and peer rejection stress sessions

Associations between cortisol and AA AUC response to performance and peer rejection stressors were investigated in 37 participants who completed both stress sessions. Associations between AUC response to performance and rejection stressors were moderate for cortisol (Spearman's ρ=.39, p<.05; ρ's=.36 and .29 for boys and girls, respectively; Figure 4a) suggestive of both consistency in response across stressors, as well as sensitivity to stressor context. Associations between AA AUC response to performance and rejection stressors were large for AA (ρ=.81, p<.001; ρ's=.85 and .78 for boys and girls, respectively; Figure 4b), suggesting a high degree of consistency in AA response across stressor domains.

Insert Figure 4 about here

4. Discussion

In this pilot study of a typically developing sample, we found sex differences in HPA (indicated by saliva cortisol) and ANS (indicated by saliva alpha amylase; AA) stress response by stressor domain and pubertal development. Girls demonstrated differing relative response to peer rejection versus performance challenges based on pubertal development, while boys did not.

Specifically, post-pubertal girls (Tanner IV-V) showed increased ANS and equivalent HPA relative response to rejection vs. performance, while pre-pubertal girls (Tanner I-III) showed heightened relative HPA and ANS response to performance vs. rejection. In contrast, pre- and post-pubertal boys showed heightened HPA and equivalent ANS response to performance vs. rejection stressors. Although results are preliminary, they suggest increases in relative sensitivity to rejection vs. performance stressors in girls but not boys across development. They also provide preliminary evidence for *female-specific malleability of stress response* across development, but *male-specific stability of stress response* across development. In the subset of participants who completed both performance and peer rejection stressors, preliminary findings revealed that HPA response showed greatest sensitivity to stressor domain, while cross-stressor consistency was greatest for the ANS response.

The present pilot study is an initial study in this area and thus results should serve primarily to point to directions for future research. Key limitations of the pilot study include: (a) the small sample size to investigate both sex and developmental differences, (b) the wide age range of participants leading to small numbers of participants at each age/pubertal stage and confounding of age and pubertal development, (c) the limited number of participants completing both stressor tasks, and (d) the cross-sectional design. Future large-scale studies utilizing longitudinal or mixed designs involving participants matched on age and differing in pubertal development and involving repeated testing of response to peer rejection and performance stressors over development are needed. Also needed are studies utilizing more sophisticated measures of pubertal development including hormonal measures, and nurse/physician Tanner staging. Despite these limitations, there are several methodological strengths, including: (a) detailed characterization of participants' stress response across 1-2, two-hour stress sessions

including multiply repeated HPA and ANS measures, (b) use of an initial "rest" session allowed physiological acclimatization to the laboratory environment prior to stress session(s), and (c) use of two developmentally relevant stressors representing contrasting stressor domains.

Study findings complement a prior investigation by our group showing increased cortisol response to peer rejection challenge in women, but increased cortisol response to achievement challenge in men [56]. The present study highlights increased relative biological sensitivity to peer rejection versus achievement challenge in post-pubertal girls, while pre-pubertal girls showed increased response to achievement vs. peer rejection challenge. In contrast, boys showed continued increased biological sensitivity to achievement versus peer rejection challenge across the pubertal transition. Findings from the present study confirm the importance of considering, measuring and manipulating stressor domain, and also including peer and relationship challenges in studies of sex differences in stress response. Numerous laboratory stressors have been termed "social"; however, most involve negative evaluation by an audience (public speaking, mental arithmetic in front of a stern audience), rather than true peer or social interactions [77, 78]. Indeed, Ellis et al. [79] have proposed that from an evolutionary perspective, social evaluative stressors involve both agonist and affiliative threat (threat to social status as well as a rejection threat).

Results also complement prior work by our group showing sex differences in cortisol response to a biological challenge (corticotropin-releasing hormone; CRH administration) over puberty in a sample of typically-developing children [80]. Girls showed increased cortisol response to CRH challenge with increasing pubertal stage, while boys showed no differences in cortisol response over puberty [80]. Results from this biological challenge study, the present pilot study and studies of basal cortisol regulation (girls>boys) [45-47] highlight the possibility

of "normative" and nuanced sex differences in HPA regulation emerging over the pubertal transition. In particular, girls appear to show increasing basal HPA set point, increasing sensitivity to HPA stimulation, and differing sensitivity to stressor domain over development, whereas boys show fewer alterations in HPA regulation over development. Given potent effects of cortisol on the brain as well as increased plasticity of the adolescent brain, even subtle alterations in basal and reactive HPA function in girls may have an impact on trajectories of brain development, particularly in circuits mediating affective and neuroendocrine response to stress [81].

The present study revealed a normative increase in relative neuroendocrine response to peer rejection versus performance challenge in girls across the pubertal transition paralleling the emergence of sex differences in depression over the same transition. Shifts in stress response in typically-developing girls may indicate increasing salience of interpersonal events and may also facilitate adaptation to interpersonal stressors, which become increasingly common for adolescent girls [82]. In high-risk adolescents, this normative shift toward biological sensitivity to peer rejection might tip the balance toward greater risk for depression following peer/romantic rejection. Indeed, several studies have demonstrated increased sensitivity to the depressogenic effects of interpersonal stressors in women and girls, but agentic/achievement stressors in men and boys [83-86]. We have previously proposed neurobiological response to peer rejection as a contextually relevant biomarker of risk for adolescent depression [57, 87]. Specifically, we have shown increased reactivity to peer rejection in a network of ventral brain regions implicated in affective processing of social information in depressed versus control youth, as well as with increasing reactivity to rejection over pubertal development [57, 87]. Prior studies have also shown peer rejection as a longitudinal predictor of depressive symptoms [88-90]. Oldehinkel

and Bouma [53] highlighted both increased malleability in response to internal and external influences and increasing sensitivity to stressful life events (especially interpersonal events) in adolescent girls, leading to improved adaptation to some circumstances but also vulnerability to depression.

The present study allowed an initial examination of cross-stressor consistency in HPA, and ANS response across performance and rejection challenges. Although significant associations were found for both AA and cortisol response to performance and peer rejection stressors, stronger associations emerged for AA (Spearman's rho=.81) than cortisol (Spearman's rho=.39). Results highlight within-participant consistency in rankings of cortisol response across stressors, but also reveal sensitivity to context/stressor domain. In contrast, ranking of AA responses to different contexts/stress domains showed higher levels of within-participant consistency, with less variance by context/stressor domain. Results complement prior studies demonstrating a strong trait-like component for diurnal and reactive variance in AA [91, 92]. Another possibility is that AA and the ANS are activated in response to a larger variety of stressors and a range of affective states, while cortisol and the HPA may require a very specific set of circumstances and affective states to be activated.

4.1. Conclusions

This pilot study revealed sex differences in relative biological sensitivity to performance versus rejection stressors across pubertal development groups, potentially indicating female-specific malleability of stress response across development, but male-specific stability of stress response across development. Specifically, we found increases in relative sensitivity to rejection vs. performance stressors in girls (a shift from heightened response to performance versus peer rejection to an equal or heightened response to rejection versus performance), but not boys

across development. Synthesizing with prior studies in the field, the present pilot study highlights the possibility of "normative" and nuanced sex differences in HPA and ANS regulation across development. Larger-scale, longitudinal studies including multiple developmentally-relevant stressor domains and with designs to tease apart effects of age and puberty are needed.

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Figure Legend

Figure 1. Timeline of performance and peer rejection stress sessions and saliva sampling.

NOTE. '' = Task duration in minutes. YIPS1-3 represents the Yale Interpersonal Stressor-Child Version Interactions 1-3. Speech represents the public speaking task; Math represents the mental arithmetic task; Tracing represents the mirror star tracing task. Minutes listed across the top of the timeline represent duration of each portion of the stress session in minutes; minutes listed along the bottom of the timeline represent exact minutes since the study start time for saliva sampling. Black rectangles showing 3" represent the amount of time to collect saliva sample between and after stress tasks. Due to prolonged nature of the cortisol response, for cortisol, saliva samples 1-2 represent baseline, 3-6 stress, and 7-9 recovery. Due to the immediacy of AA response to stress, for AA, saliva sample 1 represents baseline, 2-4 response to stress, and 5-9 recovery.

Figure 2a. Mean saliva cortisol levels over performance and peer rejection stress sessions in Pre-Pubertal (Tanner I-III) and Post-Pubertal (Tanner IV-V) girls and boys.

Figure 2b. Mean saliva alpha amylase levels over performance and peer rejection stress sessions in Pre-Pubertal (Tanner I-III) and Post-Pubertal (Tanner IV-V) girls and boys.

NOTE: Data presented are mean raw cortisol/amylase levels at each of nine time points over the stress sessions. Stressors included public speaking (speech), mental arithmetic (math) and mirror star tracing (tracing) for the performance session and three exclusion interaction challenges (Interact 1, Interact 2, Interact 3) for the peer rejection sessions. Duration of the stress period is highlighted.

Figure 3. Mean saliva cortisol and saliva alpha amylase area under the curve values in response to performance and peer rejection stressors in pre-pubertal (Tanner I-III) and post-pubertal

(Tanner IV-V) girls and boys.

NOTE. Data presented correspond with results from analyses of covariance. Areas under the curve (AUC) for saliva cortisol and saliva alpha amylase were modeled in the logarithmic scale with adjustment for baseline levels. Results are presented in the original scale for ease of visual display.

Figure 4a. Saliva Cortisol area under the curve (AUC): association between responses to performance and peer rejection stress sessions.

Figure 4b. Saliva alpha amylase area under the curve AUC): association between responses to performance and peer rejection stress sessions.

Table 1. Decomposition of 3-way interactions for saliva cortisol and alpha amylase area under the curve (AUC).

Stressor Domain by Pubertal Group[1] within Sex							
Sex	**Pubertal Group**	**Stressor Domain: Performance vs. Rejection**		**Stressor Domain X Pubertal Group Interaction**		**Stressor Domain X Pubertal Group X Sex Interaction**	
		AUC Ratio (95% CI)	*p*	*Ratio of AUC ratios (95% CI)*	*p*	*Ratio of ratios of AUC ratios (95% CI)*	*p*
CORTISOL RESPONSE							
Girls	**Pre**	2.00 (1.48-2.70)	<.001	0.46 (0.31-0.67)	<.001	0.48 (0.28-0.81)	.008
	Post	0.92 (0.73-1.14)	.439				
Boys	**Pre**	1.54 (1.14-2.09)	.007	0.96 (0.66-1.40)	.840		
	Post	1.48 (1.18-1.85)	.001				
ALPHA AMYLASE RESPONSE							
Girls	**Pre**	1.36 (1.05-1.76)	.022	0.57 (0.42-0.77)	<.001	0.61 (0.39-0.95)	.033
	Post	0.77 (0.65-0.92)	.004				
Boys	**Pre**	1.25 (0.91-1.71)	.142	0.93 (0.68-1.27)	.633		
	Post	1.16 (1.03-1.30)	.017				

Note. The pre-pubertal group (Pre) includes Tanner stages I-III; the post-pubertal group (Post) includes Tanner stages IV-V. Ratio of ratios were utilized in place of slope analyses to elucidate interactions because AUC values were calculated in the logarithmic scale then back-transformed to the original AUC scale by exponentiation.

Figure 1.

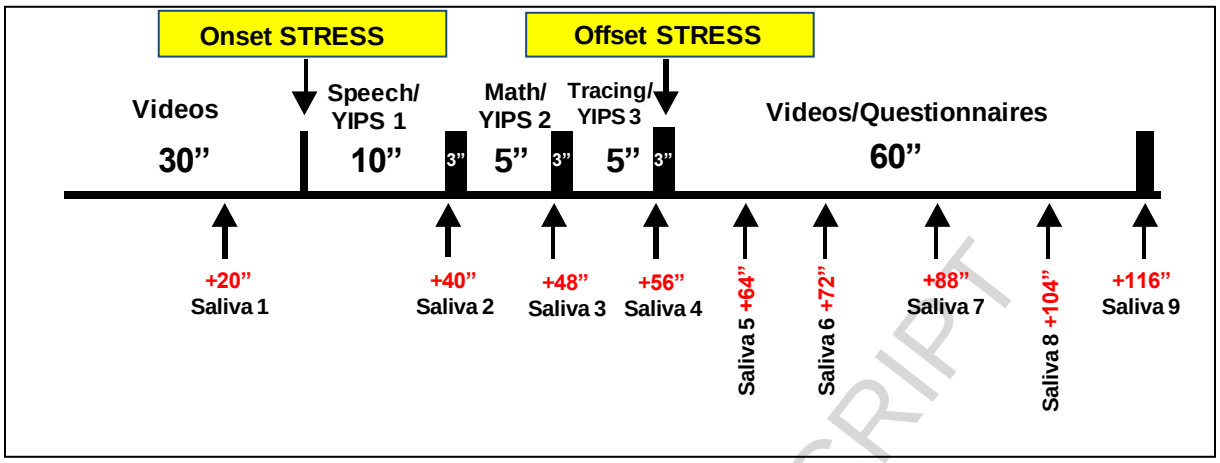


Figure 2.

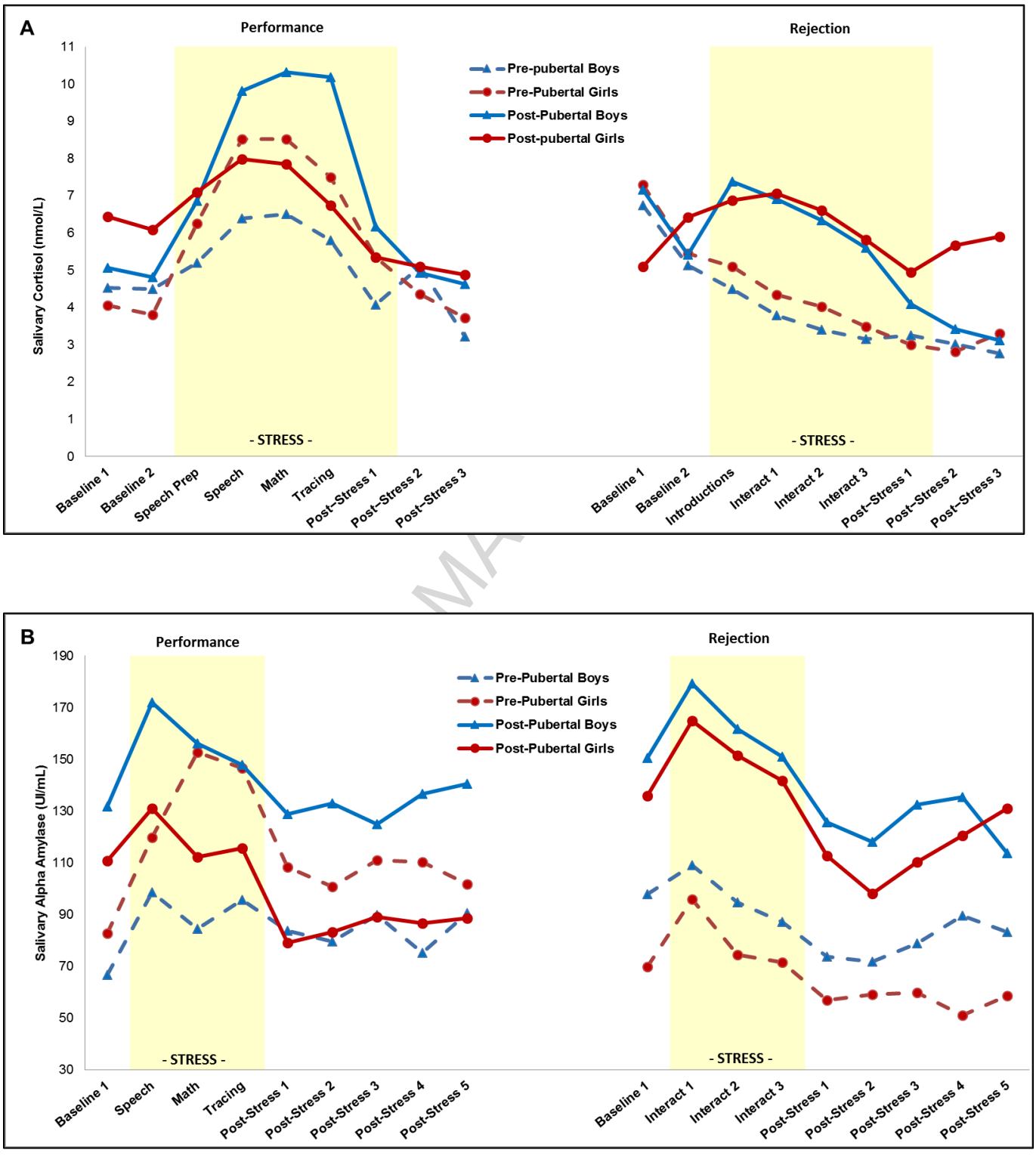


Figure 3.

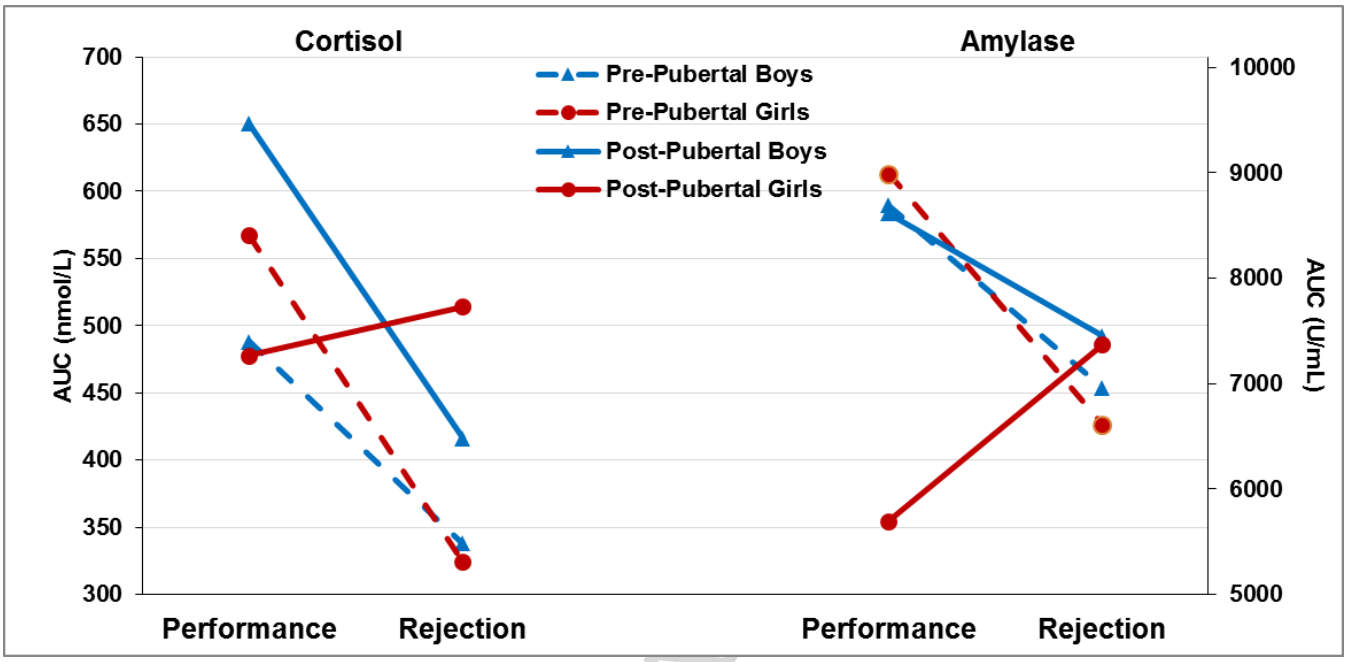
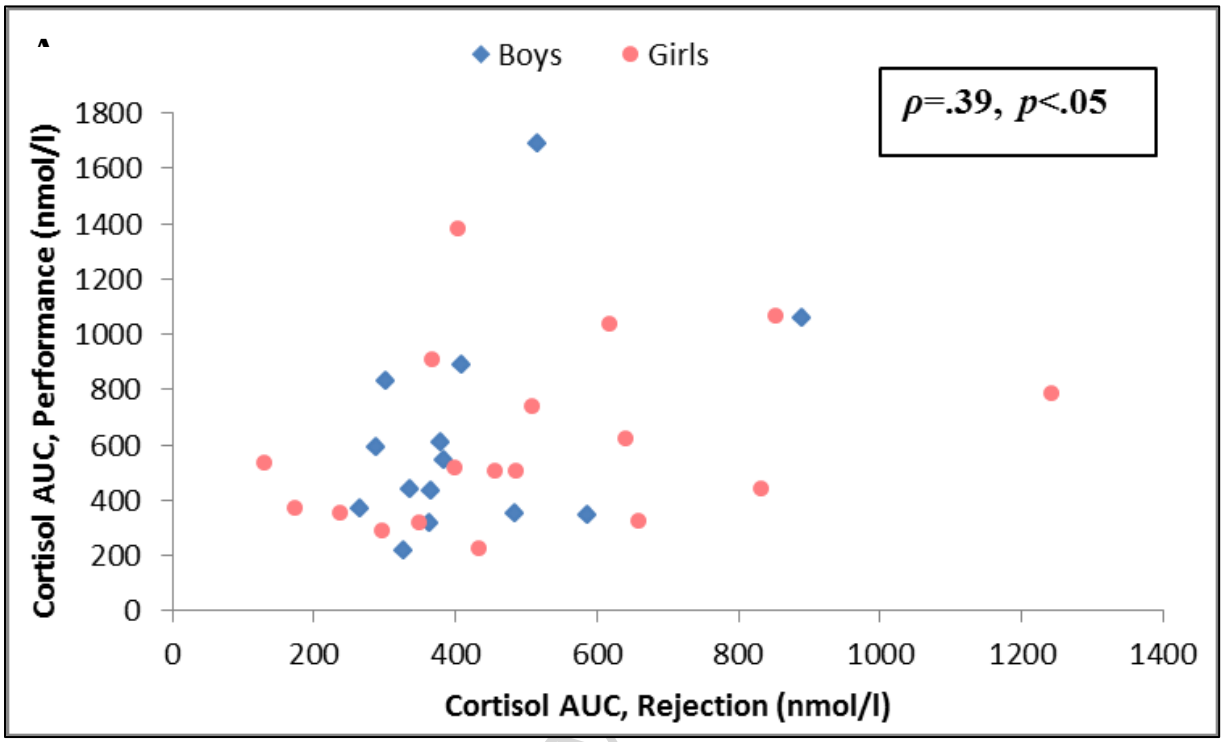
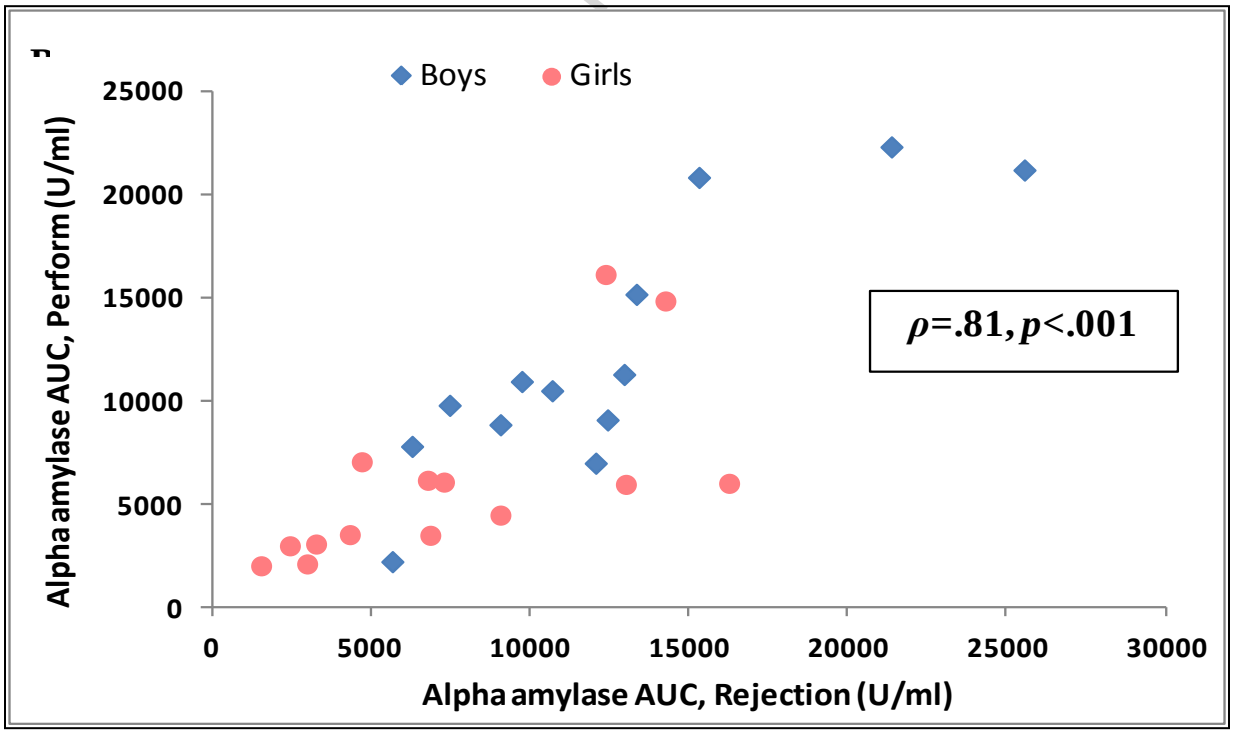


Figure 4.





HIGHLIGHTS

- Pilot study of sex differences in stress response across development.
- Older girls showed increased biological sensitivity to rejection vs. performance.
- Girls showed malleability of biological stress response across development.
- Boys showed stability of biological stress response across development.
- Results highlight the importance of future longitudinal studies.