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Authors

Marceau, Kristine
Shirtcliff, Elizabeth A
Hastings, Paul D
[et al.](#)

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Within-adolescent coupled changes in cortisol with DHEA and testosterone in response to three stressors during adolescence

Kristine Marceau^{1,2,3}, Elizabeth A. Shirtcliff⁴, Paul Hastings⁵, Bonnie Klimes-Dougan⁶, Carolyn Zahn-Waxler⁷, Lorah Dorn¹, and Elizabeth J Susman¹

¹The Pennsylvania State University

²Division of Behavior Genetics, Rhode Island Hospital Department of Psychiatry

³Brown University Center for Alcohol and Addiction Studies

⁴University of New Orleans

⁵University of California-Davis

⁶University of Minnesota

⁷University of Wisconsin

Abstract

It is hypothesized that Hypothalamic-Pituitary-Adrenal and Hypothalamic-Pituitary-Gonadal axes function together to maintain adaptive functioning during stressful situations differently in adolescence than the characteristic inverse relations found in adulthood. We examined within-person correlated changes (coupling) in cortisol, DHEA and testosterone in response to parent-adolescent conflict discussion, social performance, and venipuncture paradigms. Data are derived from two samples of boys and girls from the Northeastern US (213 adolescents aged 11–16, $M=13.7$, $SD=1.5$ years; 108 adolescents aged 9–14, $M=11.99$, $SD=1.55$) using different biological sampling vehicles (saliva and blood). Results consistently show that across samples, vehicles, and contexts, cortisol and DHEA and cortisol and testosterone are positively coupled in response to environmental stimuli. Findings underscore the importance of considering the effects of multiple hormones together in order to further our understanding of the biological underpinnings of behavior, especially during adolescence, as adolescence is a developmental transition period that may be qualitatively different from adulthood in terms of hormone functioning.

Keywords

cortisol; testosterone; DHEA; social stress; venipuncture; within-person coupling; adolescence

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Address correspondence to Kristine Marceau. Mailing address: 201 S Main St. Room 413, Center for Alcohol and Addiction Studies, Brown University, Providence, RI, 02903. Kristine_Marceau@Brown.edu. Telephone: 414-940-7380.

Kristine Marceau conducted data analysis and drafted the manuscript. Elizabeth A. Shirtcliff assisted in conceptualizing the paper and interpreting results, and provided comments on the manuscript. Bonnie Klimes-Dougan, Paul D. Hastings, and Carolyn Zahn-Waxler were involved in the data collection for Study 1, and provided comments on the manuscript. Lorah D. Dorn and Elizabeth J. Susman were involved in the data collection for Study 2, and provided comments on the manuscript.

All authors declare that they have no conflicts of interest.

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Changes in output hormones of the hypothalamic-pituitary-adrenal (HPA) axis (i.e., cortisol, dehydroepiandrosterone [DHEA]) have long been studied as predictors of psychopathology during adolescence, and an emerging literature considers hypothalamic-pituitary-gonadal (HPG) axes (i.e., testosterone) changes likewise as predictors of psychopathology symptoms, especially during adolescence. Cortisol, DHEA, and testosterone all demonstrate stress-reactive properties to various types of stressors during adolescence. However, dual-axis approaches highlight that the effects of each hormone are unlikely to influence biological processes and later behavior individually (Mastorakos et al., 2006; Rivier et al., 1986; Viau, 2002). A major gap in the literature is the lack of a basic understanding of how hormones of the HPA and HPG axis operate together across development. The present study addresses this gap by examining how cortisol, DHEA, and testosterone are associated in response to three laboratory-based stressors commonly used to assess hormone-behavior associations during adolescence.

In adults, activation in the HPA axis suppresses the activity of the HPG axis (Romeo, 2005; Stratakis and Chrousos, 1995; Terberg et al., 2009). However, adolescence is both stressful and a period of intense physical growth and development, so suppression of the HPA axis by the HPG axis or vice versa, could be developmentally inappropriate and even harmful since both systems undergo major reproductive and physical developmental changes during adolescence. Preliminary evidence reveals that a positive association between HPA and HPG hormone activity may be present in adolescents (Susman et al., 1987; Marceau et al., 2012; Matchock et al., 2007; Popma et al., 2007), potentially due to increased levels of activity in both axes during puberty (Gunnar et al., 2009; Romeo, 2005). Indeed, a small but growing group of studies in adolescents show positive associations between testosterone, DHEA, and cortisol responses to environmental stimuli (i.e., MRI: Eatough et al., 2009; exercise: Kraemer et al., 2001; venipuncture: Marceau et al., 2012). Theoretically, the ways in which these three hormones respond to stressors together (i.e. coupled responses), or separately (i.e. uncoupled) may index the hormonal milieu, or endogenous hormonal environment, and better characterize endocrine functioning. Hereafter, associations in how disparate hormones respond together is defined as coupling.

There are multiple mechanisms by which hormone responses may be coupled. Biologically, cortisol and DHEA are likely positively coupled in response to stressors because both hormones are released from the adrenal gland as part of the HPA stress response (Sapolsky, 1992, 2003), and cortisol and DHEA responses to social stress has been demonstrated to be correlated in men and women (Lennartsson et al., 2012). Evidence of the suppression of the HPG axis by the HPA axis through inverse relations between cortisol and testosterone suggests that testosterone and cortisol would be inversely coupled in adults. However, positive associations between cortisol and testosterone and cortisol and DHEA in adolescents suggest that we may find positive coupling in adolescents, potentially due to increased activation of both axes generally due to puberty (Marceau et al., accepted). From an environmental perspective, while different types of environmental stimuli precipitate a release in testosterone and in cortisol, there is some evidence of individual differences in the extent to which the same environmental cue precipitates the recruitment of multiple hormones (e.g., Eatough et al., 2009; Kraemer et al., 2001; Marceau et al., 2012). Considered simultaneously, the three hormones examined here could comprise a more comprehensive biomarker during adolescence than examining one hormone at a time (see Marceau et al., accepted for review).

Hormonal Milieu

Multiple hormones may impact biological underpinnings of behavior such that together they maintain allostasis, or the body's ability to adapt and regulate to changing environmental

challenges (Sterling and Eyer, 1988). Given the observed negative associations between hormones of the HPA and HPG axes in adults (Viau, 2002), it is reasonable to hypothesize that the HPA and HPG axes are implicated in counter-regulation mechanisms maintaining allostasis, just as multiple neuroendocrine systems have been implicated in counter-regulation mechanisms in drug addiction (e.g., Koob and Le Moal, 2001). Thus, examining multiple hormones acting in concert may be more fruitful than examining single hormones: the influence of multiple hormones on behavior may be a better index of the complex system represented by these hormones.

There is a brief history of considering how hormones of the HPA and HPG axes are together associated with behavior. Much of the literature examining associations of multiple hormones on behavior has used cortisol-DHEA (e.g., Goodyer et al. 1998; Goodyer et al., 2003; Izawa et al., 2008; Izawa et al. 2012; Young et al., 2002) and testosterone-cortisol ratios (e.g., Van Honk et al., 2010, Montoya et al., 2012; Terburg et al., 2009). According to the cortisol-DHEA ratio hypothesis, DHEA balances cortisol and buffers the body from harmful effects of prolonged exposure to cortisol (e.g., Herbert, 1997; Herbert, 1998; Kimonides et al., 1998; Mao & Barger, 1998), so a high cortisol-to-DHEA ratio indicates an imbalance that, for example, may predispose individuals to psychopathology or diverse behavior problems (Goodyer et al., 1998; Goodyer et al., 2003).

According to the testosterone-cortisol ratio hypothesis, high testosterone in the presence of low cortisol is associated with aggression and externalizing problems in adults through up-regulation of gene expression in several brain regions including the amygdala (Van Honk et al., 2010; Montoya et al., 2012). Similarly, higher levels of testosterone predicted overt aggression in boys and men who had low cortisol levels, but not in boys who had high cortisol levels (Dabbs et al., 1991; Popma et al., 2007; Mehta and Josephs, 2010; see Montoya et al., 2011). Studies testing the testosterone-cortisol ratio have included mainly adults and suggest that elevated testosterone levels in the context of impaired adrenal responses may be characteristic of the “fight” response. Notably, these studies examine levels of each hormone, rather than responses of multiple hormones to specific stressors. One study found that increased testosterone-to-cortisol responsivity (in response to a countdown/unannounced loud noise task and the Trier Social Stress Test assessing uncontrollability and social evaluative threat) ratios were associated with psychopathy among adults (Glenn et al. 2011) but these findings have not been replicated in youth.

This literature highlights between-person differences in cortisol-DHEA and testosterone-cortisol ratios in relation to behavioral correlates. However, previous studies highlight the importance of separating between- from within- person changes in predictors of behavior, as between and within-person changes are distinct constructs (e.g., Ram and Gerstorf, 2009; Nesslerode, 1991), and because between-and within-person changes in hormones have been shown to differentially predict behavior (e.g., Kraemer et al., 1976, see also Marceau et al., in press). Here, we propose that taking a within-person approach examining the extent to which hormone responses to stressors are coupled or uncoupled within-individuals would better index individuals' hormonal milieus.

Contextual Moderators

Biosocial theories posit that physiological profiles, usually stress response profiles measured via cortisol, are not inherently adaptive or maladaptive, but that the effects of hormone levels and responses depend on contextual factors (Ellis et al., 2011). The first contextual factor to consider when examining stress responsivity of hormones is the stressor itself. There is some evidence that stress responsivity of testosterone, DHEA, and cortisol may differ by context. For example, cortisol is responsive to multiple laboratory stressors,

especially when stressors are seen as uncontrollable (see Dickerson and Kemeny, 2004 for meta-analysis and review). It has been hypothesized that DHEA may be responsive to frustration within social challenges in particular (e.g., Marceau, et al., accepted). DHEA was responsive to a parent-child conflict interaction task during adolescence (Shirtcliff et al., 2007), has been shown to respond to the Trier Social Stress Test (Lennartsson et al., 2012), and increased during an impossible anagram task that elicited frustration (Wemm et al., 2013). Testosterone appears to increase in response to threat (Sapolsky, 1982), competitive challenges and strenuous exercise (Bateup et al., 2002; Kraemer et al., 2001; Sutton et al., 1973; Volek et al., 1997; see Zitzmann and Nieschlag, 2001 for review). However, mental stresses (e.g. anticipating exams, workplace reorganization), as opposed to physical stresses, may decrease testosterone levels (Zitmann and Nieschlag, 2001). The release of the mental stress (e.g. removal of workplace stress) may lead to a subsequent rise in testosterone (Grossi et al., 1999). Taken together, different types or aspects of stressors may elicit responses from one or more of these three hormones. A small group of studies have examined responses of each of these hormones to the same stressor, and consistently show substantial individual variability in hormone responses – some adolescents respond to the same stressor with all three hormones, whereas others do not (Eatough et al., 2009; Kraemer et al., 2001; Marceau et al., 2012). Therefore, associations between HPA and HPG hormones may not be uniform for every type or interpretation of the stressor.

Present Study

We used secondary data from two studies to examine within-person coupling of cortisol with DHEA and testosterone responsivity to environmental stimuli across three different contexts: a parent-adolescent conflict discussion paradigm, a social performance paradigm, and a venipuncture paradigm. Because of age and sex differences in hormonal changes during adolescence, we included age¹ and sex as moderators of within-person coupling. We hypothesized that there would generally be positive associations between cortisol, DHEA, and testosterone responsivity within-individuals following the few studies showing between-person associations in these hormones during adolescence, with intraindividual differences in the strength of these associations. We made no directional hypotheses of moderators of within-person associations.

Study 1

Method

Participants and Procedures—In the first study, participants were 106 adolescent boys and 107 adolescent girls aged 11–16 ($M = 13.7$, $SD = 1.5$ years), and their parents who participated in a longitudinal investigation of the role of emotion in the development of psychopathology (Adolescent Emotion Study, AES, Klimes-Dougan et al., 2001). Participants were recruited through announcements in newspapers and flyers in the Washington DC metropolitan area. Potential participants were administered a telephone screening including an abbreviated version of the Child Behavior Checklist (A-CBCL, Achenbach, 1991), and the full checklist at a laboratory visit. Adolescents were oversampled for internalizing and externalizing psychopathology: approximately 1/3 of the adolescents were in the normal range of problems (T scores < 60), 1/3 had sub-clinical problems (T scores between 60 and 63, and 1/3 had clinical problems (T scores > 63). Participants were balanced during recruitment for approximately equal proportions of youth with internalizing, externalizing, and comorbid internalizing and externalizing psychopathology among those

¹ Pubertal stage was also considered as a moderator of coupling in lieu of age, but had no systematic effect on coupling beyond what was observed with age. Age was used in the current analysis as it was more robust.

with sub-clinical and clinical levels of psychopathology (for additional sampling and recruitment information see Klimes-Dougan et al., 2001).

After screening, participation followed several steps. First, parental consent and child assent was collected at the start of the home visit, where observational, self-report, and biological data were collected. Participants filled out self-report questionnaires and provided biological data between the home visit and laboratory visit. Two-to-three weeks after the home visit participants visited the laboratory and diagnostic, observational, and biological data was collected. Relevant to the current study, families participated in a conflict discussion paradigm (CDP) at the home visit and a social performance paradigm (SPP) at the laboratory visit (described below). Saliva was collected prior to each discussion (CDP: $M = 4:20\text{pm}$, $SD = 1\text{hr } 43\text{min}$; SPP: $M = 11:45\text{am}$, $SD = 56\text{ min}$), and approximately 20 and 40 minutes after the stressor surrounding each task.

SPP—The SPP occurred during a laboratory visit occurring in the late morning. Families arrived at the laboratory at approximately 8:30am, and the SPP occurred approximately 2 hours and 15 minutes later (after participants filled out questionnaires and had a 15 minute break). The SPP was a 6-minute task where adolescents were first asked to carry on a conversation with an unfamiliar “shy” female staff member for 3 minutes, who gave only brief answers to questions and did not initiate questions. Then, adolescents were instructed to give a 3-minute speech describing themselves and their school (with one minute to prepare). The two audience members were instructed to provide only minimal feedback.

CDP—The CDP was a series of discussions occurring during a home visit. The mother-youth CDP always preceded the father-youth CDP in families with participating fathers; only the mother-youth CDP was used in the current report because saliva was not collected surrounding the father-youth CDP. The first 3-min discussion was a warm-up task wherein dyads planned an all-expense paid vacation together. The second 3-min discussion was designed to assess how the dyad discussed internalizing emotions. Dyads discussed a time when the adolescent was sad or worried. Finally, the dyad completed a 6-minute task designed to elicit conflict, in which the mother and adolescent were asked to discuss a topic they had each independently previously reported as a topic from an issues checklist about which they frequently had intense conflict.

Measures—In the AES sample, three saliva samples were collected in response to each task (CDP and SPP) – at baseline (preceding the stressor, 0 minutes), approximately 20 after the stressor, and approximately 40 minutes post-stressor. Testosterone, DHEA, and cortisol were assayed from saliva collected through passively drooling approximately 5 ml of saliva into a test tube, supervised by research assistants. Participants did not eat during the 30 minutes prior to each saliva collection. The saliva was stored in the tubes at -25°C . After being shipped overnight on dry ice to The Pennsylvania State University Behavioral Endocrinology Laboratory, saliva was stored at -86°C until assayed with Salimetrics' Enzyme Immunoassay in duplicate. Measures of each were reliable; detailed information on assays and collection times for this sample can be found elsewhere (Granger et al., 2003; Klimes-Dougan et al., 2001; Shirtcliff et al., 2007). Means and standard deviations of the raw hormone levels at each collection are presented in Table 1. Youth with hormone levels over 2.5 SD of the sample mean for boys and girls separately were winsorized to 2.5 SD values (< 5% of cases across samples and hormones), and then scores were log-transformed to normalize the distribution. For each hormone, some youth increased from 0–20 minutes only (CDP cortisol: 10%; SPP cortisol: 38%; CDP DHEA: 19%, SPP DHEA 40%; CDP testosterone: 22%; SPP testosterone: 31%) and some youth's hormones increased from 20–40 minutes only (CDP cortisol: 23%; SPP cortisol: 19%; CDP DHEA: 16%, SPP DHEA 17%; CDP testosterone: 30%; SPP testosterone: 25%), and finally some youth's hormones

increased across both time intervals (CDP cortisol: 4%; SPP cortisol: 12%; CDP DHEA: 8%, SPP DHEA: 24%; CDP testosterone: 6%; SPP testosterone: 15%). For some youth, hormones decreased across the 40 minutes (CDP cortisol: 63%; SPP cortisol: 31%; CDP DHEA: 47%, SPP DHEA 21%; CDP testosterone: 42%; SPP testosterone: 29%).

Analytic Strategy

Missing Data—Missing data were imputed using SAS PROC MI; 40 datasets were imputed and aggregated (Graham et al., 2007) to reduce bias. Less than 12% of the data were missing any hormone assessment, or age or sex in either study.

Within-person Coupling of Responsivity to Environmental Stimuli—In order to understand within-person coupling of hormones, we examined how changes in cortisol and changes in DHEA and testosterone in response to three stressors were associated within-adolescents, without imposing a presupposed shape of change (see below). Specifically, we tested for coupling (i.e., correlated changes within each adolescent of cortisol and DHEA and cortisol and testosterone) in response to the conflict discussion paradigm (CDP) and the social performance paradigm (SPP) in the AES, and to the venipuncture paradigm in Study 2 (NIMH-NICHD, below).

Data preparation: All analyses were conducted using SAS PROC MIXED. It is important to separate differences in overall hormone functioning (i.e., between-person differences, or the between-person portion of the variance of hormone change – extent to which individuals differ from each other generally) from within-person changes (i.e., within-person portion of the variance in hormone change – or how much an individual changes over time compared with his/her own typical hormone levels) to ensure that associations between changes in hormones are not driven by both hormones being higher or lower in some individuals than others within the sample. In order to ensure that our coupling parameters represented within-person coupling in response to the stress paradigms, unconfounded by differences in mean levels of each hormone, we conducted no-growth, unconditional mixed effects models for DHEA and testosterone separately in order to separate each individual's average hormone level from the collection-to-collection variation in response to each environmental stimuli (i.e., removing the time-varying structure of the data). The *average* instead of *basal* level was extracted because we did not impose a structured shape of change (for example, by including time in the model and estimating a linear slope). We chose this method because of the vast individual differences in response patterns to these tasks (Klimes-Dougan et al., 2001; Marceau et al., 2012); any specified shape of change would not fit the data well for substantial proportions of the samples. Therefore, in the final models we were able to test if one hormone was relatively higher (or lower) within an individual when the other hormone was also relatively higher (or lower) within that same individual, controlling on any overall between-person differences in levels of functioning.

Specifically, we extracted the empirical Bayes estimate for each individual's average DHEA and testosterone score across the three collections in response to each stressor (DHEA level and testosterone level). This is a measure of each individual's average hormone level across the task, and is a measure of between-person differences in overall hormone levels. We also extracted the empirical Bayes estimates for each individuals' residuals at each collection (i.e., 0 minutes, 20 minutes, 40 minutes), which describe the extent to which the hormone level was above or below each individual's average score at each of the three collections (referred to here as DHEA change and testosterone change) to be used in hypothesis testing. The mean and residual scores were used as separate predictors of cortisol in coupling models (described below).

Hypothesis testing: In order to test hypotheses about within-person coupling of hormones, we conducted a second set of mixed effects models. Two bivariate models were conducted: 1) DHEA predicting cortisol, and 2) testosterone predicting cortisol. Then, a parallel trivariate model was conducted with DHEA and testosterone both predicting cortisol in the same model. Bivariate models assessed coupling between two hormones (i.e. cortisol with DHEA and testosterone separately). However, in the trivariate model, coupling of cortisol and DHEA was tested while controlling on the extent to which cortisol and testosterone are coupled, and coupling of cortisol and testosterone was tested while controlling on the extent to which cortisol and DHEA are coupled. The result is that coupling between cortisol and DHEA represents coupling of purely adrenal hormones, as any overlapping variance between DHEA coupling and testosterone coupling due to gonadal maturation is controlled for by adding testosterone. Likewise, coupling between cortisol and testosterone represents coupling between the HPA and HPG axes. The first bivariate model is described below.

Level 1:

$$\text{Cortisol}_{ti} = \beta_{0i} + \beta_{1i} (\text{DHEA response}_{ti}) + e_{ti} \quad (2)$$

Level 2:

$$\begin{aligned} \beta_{0i} &= \gamma_{00} + \gamma_{01} (\text{DHEA level}) + \gamma_{02} (\text{Age}_i) + \gamma_{03} (\text{Sex}_i) + \gamma_{04} (\text{Age}_i * \text{Sex}_i) + u_{0i} \\ \beta_{1i} &= \gamma_{10} + \gamma_{11} (\text{Age}_i) + \gamma_{12} (\text{Sex}_i) + \gamma_{13} (\text{Age}_i * \text{Sex}_i) + u_{1i} \end{aligned}$$

where Cortisol_{ti} is individual i 's cortisol level at time t , β_{0i} is the intercept term indicating the predicted level of cortisol at the mean level of DHEA, β_{1i} is the coefficient describing the coupling between changes in cortisol and changes in DHEA from 0–40 minutes post-stressor, and e_{ti} contains individual i 's residual errors.

At level two, γ_{00} is the intercept term indicating the predicted score of cortisol at the initial level of DHEA at the sample mean of between-person predictors; γ_{10} is the coupling parameter at the sample mean of between-person predictors. γ_{01} is the coefficient describing the extent to which initial levels of cortisol are moderated by overall levels of DHEA (which controls on between-person associations between cortisol and DHEA levels), γ_{02} is the coefficient describing the extent to which initial levels of cortisol are moderated by age, γ_{03} is the coefficient describing the extent to which initial levels of cortisol are moderated by sex, γ_{04} is the coefficient describing the extent to which initial levels of cortisol are moderated by the interaction of age and sex, and u_{0i} describes the individual variations around the initial levels of cortisol. Finally, γ_{11} is the coefficient describing the extent to which age moderated the coupling between cortisol and DHEA, and γ_{12} is the coefficient describing the extent to which sex moderated the coupling between cortisol and DHEA, γ_{13} is the coefficient describing the extent to which the interaction of age and sex moderated the coupling between cortisol and DHEA, and u_{1i} describes the individual variations around the coupling parameter. We did not hypothesize that DHEA level would moderate the extent of within-person cortisol-DHEA coupling, and therefore we did not include it in the model.

Results

Results for bivariate models of coupling between cortisol and DHEA are presented in Table 3. Key results are discussed in text. In both the CDP and SPP, cortisol and DHEA were positively coupled, CDP: $\gamma_{10} = .04$, $SE = .008$, $p < .05$; SPP: $\gamma_{10} = .03$, $SE = .008$, $p < .05$. Age moderated the extent to which cortisol and DHEA were coupled in response to the SPP only, such that there was tighter coupling among older relative to younger adolescents, γ_{11}

= .004, SE = .002, $p < .05$. Further, the interaction of age and sex also moderated the extent to which cortisol and DHEA were coupled in response to the SPP only, $\gamma_{13} = -.01$, SE = .003, $p < .05$, such that coupling was stronger among older boys than among younger boys, but there were not significant age differences in coupling among girls. There was significant individual variability in cortisol-DHEA coupling in the CDP $u_i = .0004$, SE = .0001, $p < .05$ but not the SPP, $u_i = .0001$, SE = .0001, $p < .05$. Results for the bivariate models of coupling between cortisol and testosterone are presented in Table 4. Cortisol and testosterone were positively coupled in the CDP and SPP, CDP: $\gamma_{10} = .04$, SE = .01, $p < .05$; SPP: $\gamma_{10} = .04$, SE = .01, $p < .05$. Age moderated the extent to which cortisol and testosterone were coupled in response to the CDP only, such that coupling was stronger among younger adolescence and weaker among older adolescents, $\gamma_{11} = -.02$, SE = .008, $p < .05$. There was significant individual variability in cortisol-testosterone coupling in both the CDP and SPP, $u_i > .0007$, SE < .0003, $p < .05$.

Results for the trivariate models of coupling are presented in Table 5. Paralleling results from the bivariate model, cortisol and DHEA were positively coupled in the CDP and SPP, $\gamma_{10} > .03$, SE < .008, $p < .05$. However, unlike the bivariate model, cortisol-testosterone coupling was not significant during the CDP in the trivariate model, $\gamma_{20} = .02$, SE = .01, $p > .05$. As in the bivariate models, cortisol and testosterone were positively coupled during the SPP in the trivariate model, $\gamma_{20} = .03$, SE = .014, $p < .05$. Additionally, the effect of age on cortisol-DHEA coupling observed in the bivariate model for the SPP was also significant for the CDP: older adolescents had tighter cortisol-DHEA coupling in the CDP and SPP after controlling for cortisol-testosterone coupling, γ_{11} 's > .01, SEs = .005, p 's < .05. As in the bivariate model, the interaction of age and sex also moderated the extent to which cortisol and DHEA were coupled in response to the SPP, such that coupling was stronger among older boys than among younger boys, but there were not age differences in coupling among girls, and this effect was also present for the CDP in the trivariate model, $\gamma_{13} = -.008$, SE = .003, $p < .05$. In the trivariate model there were addition effects of age, $\gamma_{11} = -.02$, SE = .008, $p < .05$, and the age by sex interaction, $\gamma_{13} = .01$, SE = .005, $p < .05$, on cortisol-testosterone coupling in response to the CDP only, such that younger boys had stronger positive cross-axis coupling than younger girls, but the effect was not found among older adolescents. In other words, the age effect suggesting that younger adolescents had stronger cross-axis coupling than older adolescents was driven by boys. There was significant individual variability in cortisol-DHEA coupling in the CDP $u_i = .0004$, SE = .0001, $p < .05$ but not the SPP, $u_i = .0001$, SE = .0002, $p < .05$. and there was significant cortisol-testosterone coupling in both the CDP and SPP, u_i 's > .0007, SEs = .0003, p 's < .05.

Study 2

Method

Participants and Procedures—In the second study, participants were 56 boys (aged 10–14, $M=12.72$, $SD = 1.32$) and 52 girls (aged 9–14, $M=11.99$, $SD = 1.55$ years) and their parents drawn from a longitudinal study of developmental changes in hormones, physical stature, and behavior during early adolescence (NIMH-NICHD; Susman et al., 1987) assessed every six months over the course of one year (3 times). Families were all intact and middle to upper-middle class, and youth were recruited to represent the full range of stages of physical maturation at baseline. For additional, detailed information on the sample and recruitment see Susman and colleagues (1987, 1991).

At each wave of data collection, adolescents and their parents participated in a 4-hour homelike laboratory visit in the early evening when they completed interviews and questionnaires. A few days later ($M = 2.3$ days) each adolescent came to a large research hospital at 8:00am. The purpose of the second visit was to obtain blood for steroid hormone

analysis and to conduct a physical examination for pubertal staging. Each adolescent underwent a venipuncture procedure with the parent present. A needle was inserted at 0 minutes, when blood was drawn, and a heparin lock was used to allow for drawing blood samples at 20 and 40 minutes, to avoid multiple punctures. Thus, the initial venipuncture procedure was the stressor.

Measures—In the NIMH-NICHD sample, 15mL blood samples were drawn at 0, 20, and 40 minutes into the laboratory visit at each six-month assessment. Cortisol, DHEA, and testosterone were reliably assayed from the blood sample (See Nottlemann et al., 1987; Susman et al., 1991 for radioimmunoassay information). Although assays using blood and saliva both assess circulating levels of hormones, the actual levels of cortisol, testosterone, and DHEA in circulation differ in blood versus in saliva (Dorn et al., 2006; Dorn & Biro, 2011). In blood samples, total cortisol, testosterone, and DHEA are measured (free plus bound) and in saliva only free (bioavailable) hormones are measured. Means and standard deviations of the non-transformed hormone levels are presented in Table 2. As in the AES sample, youth with hormone levels over 2.5 SD of the sample mean for boys and girls separately were winsorized to 2.5 SD values (< 4.6% of cases across samples, hormones, and assessments), and then scores were log-transformed. There was variation in response patterns. For each hormone, some youth's hormones increased from 0–20 minutes only (T1 cortisol: 34% of youth; T2 cortisol: 31% T3 cortisol: 29%; T1 DHEA: 36%, T2 DHEA 43%, T3 DHEA 34%; T1 testosterone: 33%; T2 testosterone: 27%, T3 testosterone: 32%). Some youth's hormones increased from 20–40 minutes only (T1 cortisol: 26%; T2 cortisol: 10% T3 cortisol: 7%; T1 DHEA: 16%, T2 DHEA 17%, T3 DHEA 20%; T1 testosterone: 35%; T2 testosterone: 34%, T3 testosterone: 32%). Some youth's hormones increased across the entire 40 minutes (T1 cortisol: 14%; T2 cortisol: 8% T3 cortisol: 6%; T1 DHEA: 22%, T2 DHEA 13%, T3 DHEA 9%; T1 testosterone: 6%; T2 testosterone: 11%, T3 testosterone: 14%). For some youth, hormones decreased across the entire 40 minutes (T1 cortisol: 26%; T2 cortisol: 52% T3 cortisol: 59%; T1 DHEA: 26%, T2 DHEA 27%, T3 DHEA 37%; T1 testosterone: 26%; T2 testosterone: 28%, T3 testosterone: 22%).

Analytic Strategy

Identical data analytic procedures were used in both studies for handling missing data, data preparation, and hypothesis testing, with one major difference. In Study 1, a single age for each individual was used. However, in the NIMH-NICHD sample there were three waves of data collection. Therefore, wave of data collection was entered as an additional random effect in hypothesis testing analyses in order to test whether there were individual differences in cortisol levels or coupling parameters over time.

Results

Results for bivariate models of coupling between cortisol and DHEA are presented in Table 3. Again, key findings are discussed in the text. As in Study 1, cortisol and DHEA were positively coupled in response to venipuncture, $\gamma_{10} = .25$, $SE = .11$, $p < .05$. As in Study 1, there was significant individual variability in cortisol-DHEA coupling in response to venipuncture when collapsing across assessments, $u_{1i} = .04$, $SE = .02$, $p < .05$. There was not significant individual variability in cortisol-DHEA coupling in response to the venipuncture paradigm across assessments in the NIMH-NICHD study, $u_{3i} < .0001$, $SE < .0001$, $p > .05$. Therefore, there was no evidence that coupling within-individuals changed over time.

Results for the bivariate models of coupling between cortisol and testosterone are presented in Table 4. Unlike Study 1, there was not a main effect of cortisol-testosterone coupling in response to venipuncture, $\gamma_{10} = -.25$, $SE = .16$, $p > .05$. However, there was evidence that

sex moderated cortisol/testosterone coupling in the venipuncture paradigm such that boys showed stronger positive coupling of cortisol and testosterone than girls $\gamma_{12} = .26$, $SE = .11$, $p < .05$. As in Study 1, there was significant individual variability in cortisol-testosterone coupling in response to venipuncture when collapsing across assessments, $u_{1i} = .04$, $SE = .03$, $p < .05$. There was not significant individual variability in cortisol-testosterone coupling to the venipuncture paradigm across assessments in the NIMH-NICHD study, $u_{3i} < .0001$, $SE < .0001$, $p > .05$.

Results for the trivariate models of coupling are presented in Table 5. Paralleling results from the bivariate model and Study 1, cortisol and DHEA were positively coupled in response to venipuncture, $\gamma_{10} = .29$, $SE = .13$, $p < .05$. As in the bivariate model, cortisol and testosterone were not coupled during the venipuncture paradigm, $\gamma_{20} = -.03$, $SE = .13$, $p > .05$. However, the effect of sex on cortisol-testosterone coupling was not significant after controlling for cortisol-DHEA coupling for the venipuncture paradigm, $\gamma_{12} = .12$, $SE = .09$, $p > .05$. There was not significant individual variability in cortisol-DHEA or cortisol-testosterone coupling (collapsing across assessments) in response to the venipuncture paradigm (contrary to the bivariate models), u_i 's $> .02$, $SEs > .04$, p 's $> .05$. As in the bivariate models, there was not significant individual variability in cortisol-DHEA or cortisol-testosterone coupling to the venipuncture paradigm across assessments in the NIMH-NICHD study, u_i 's $> .02$, $SEs < .06$, p 's $> .05$.

Discussion

In the present study we examined cross-axis and within-axis associations of cortisol, testosterone, and DHEA in response to stressors in adolescents. Specifically, we examined within-person coupling and contextual moderators of within-person coupling of cortisol and DHEA and cortisol and testosterone responses to three different stressors across two different studies and two different hormone sampling techniques, saliva and serum. Generally, there was positive coupling between cortisol and DHEA across tasks. For coupling between cortisol and testosterone, positive coupling was found for the conflict discussion paradigm and for the social performance task. This coupling pattern was also found for the venipuncture paradigm, though it was stronger for boys than for girls. The consistency in findings was remarkable. Therefore, our findings suggest that the axes operate dynamically within diverse acute settings during adolescence.

Contextual Moderators

Age moderated coupling between cortisol and DHEA in response to the SPP, and in response to the CDP when controlling on cortisol-testosterone coupling. This finding is similar to a recent longitudinal study showing that the positive coupling of basal cortisol and DHEA increased from age 11 to age 15 (Ruttle et al., 2013). Older adolescents had tighter coupling of cortisol and DHEA in response to both the conflict discussion and social performance paradigm particularly when controlling on testosterone, and therefore examining coupling of purely HPA-axis hormones, older adolescents had tighter coupling of cortisol and DHEA in response to both the conflict discussion and social performance paradigm. Age by sex interactions clarified that these age findings were driven by boys such that older boys generally had the strongest positive coupling of HPA axis output hormones. Particularly interesting was that the age effect and age by sex interaction was not present in response to the CDP until cortisol-testosterone coupling was taken into account. This suggests that in response to the CDP, coupling is specifically within-axis coupling, that is, when DHEA is secreted in response to a stressor, operating as a stress hormone rather than as a puberty-related hormone. DHEA traditionally has been considered a pubertal, or adrenarche, hormone response for androgenization of males and females. Although these

effects are cross-sectional, it may be that as adolescents age, and become more proficient at dealing with social situations, they are able to recruit DHEA to buffer from the deleterious effects of higher cortisol. These cross-sectional developmental associations found during responses to stressors appears specific to stressors involving a social component. Thus, our findings suggest that across adolescence, the experiences boys have with parents and in other social situations (e.g. at school) may contribute to how they perceive social cues and how they respond physiologically.

In post-hoc tests we examined whether pubertal maturation drove these findings (data available upon author request), but the age effects presented here generally did not hold when examining pubertal maturation, also paralleling findings from Ruttle et al., (2013). Thus, the strengthening of coupling between cortisol and DHEA across adolescence appears as a robust effect that spans from basal levels to responses to social stimuli. Pubertal maturation was measured in terms of Tanner stages: Study 1 included parent- and youth-report using Tanner pictures, and Study 2 included nurse reported Tanner stages, detailed information available upon author request). There was substantial variability in pubertal stage in both sexes in both studies (see Susman et al., 1987; Natsuaki et al., 2009). Because age but not pubertal maturation moderated coupling, it may be that social changes related to aging, or other aspects of aging during adolescence not related to puberty (i.e., brain maturation related to age but not puberty, Brenhouse & Anderson, 2011) are more likely mechanisms of the shift in hormone coupling than physiological changes due to puberty, though there may still be a smaller role for pubertal changes as well.

We also found that cortisol-testosterone coupling was weaker among older adolescents than younger adolescents, particularly for boys. This effect also echoes findings from Ruttle et al., (2013) that basal cortisol and testosterone were positively coupled at age 11 but progressed to negative coupling by age 15 (Ruttle et al., 2013). There are several very notable differences in findings: here the effect of coupling weakened, but the shift to negative coupling was not observed, and this effect was specific to boys. Nonetheless, it is remarkable that the cross-sectional findings for responsivity of cortisol and testosterone reflect the longitudinal findings of basal morning levels. Further, the effect that boys had stronger coupling between cortisol and testosterone relative to girls in response to venipuncture was paralleled in the age by sex moderation findings in the CDP. In the case of cortisol and testosterone, it may be that younger boys recruit both hormones together as they are less adept at understanding when difficult social situations are challenging in terms of social status vs. social evaluation. The perception of socially evaluative cues as related to social status may be reflected by similar responses of both cortisol and testosterone.

Hormonal Milieu

The finding that cortisol and testosterone were positively coupled in response to all three stressors is particularly interesting. Testosterone has been shown to be responsive to a variety of stressors (e.g., Eatough et al., 2009; Kraemer et al., 2001; Zitmann and Nieschlag, 2001), including in one of the current samples (e.g., Marceau, et al., 2012). The findings presented here suggest that physiologically the endocrine systems of adolescents can recruit multiple hormones to deal with a variety of stressors.

It has been hypothesized that differences in reactivity of cortisol, DHEA, and testosterone may be context-dependent, such that instead of eliciting “stress” each hormone responds to unique aspects of the environmental stimuli. However, to provide a stressor that elicits hormone reactivity in psychological studies, reactivity tasks are often employed that are intentionally multifaceted in the challenges they present. These reactivity tasks are designed to test the regulation of an individual’s coping resources (similar to what a stressor that occurs in everyday life may do); in turn, multi-level physiological changes are likely to

occur in response to such novel, uncontrollable, and socially evaluative threats (Hastings et al., 2011). These challenges may actually increase the likelihood that multiple hormones would be activated within the same task. For example, induced stressors may be construed by the adolescent as competitive or socially evocative, therefore eliciting a testosterone response, and/or as frustrating and therefore eliciting a DHEA response (e.g., Wemm et al., 2013). Positive coupling for cortisol and DHEA and cortisol and testosterone in response to these three stressors implies that stressors are not clear cut for adolescents, and that adolescents may interpret the same stressors as simultaneously stressful, frustrating, and an opportunity for a gain in social status. Thus, even when interested in reactivity within a single axis, the mechanisms that may be underlying change in the other axes should be considered as well, as activation of one axis not only changes how the individual interprets their social context, but indeed changes the very nature of their internal hormonal milieu. Further, while future studies are unlikely to be able to design psychologically-oriented stressors that activate one axis at a time, it may be possible to experimentally invoke greater degrees of frustration, unpredictability, or social-evaluative threat (e.g., Bosch et al., 2009; Het et al., 2009) to examine individual differences and test whether the positive coupling of these hormones can be influenced. As there would also be individual differences in the extent to which stressors would be frustrating, unpredictable, or stressful, perhaps individually-tailored paradigms whereby the stressor is matched to the subject in order to distinguish “real” demands of the task from the subjective experience of the task could be developed. It may be that the extent of coupling in various situations, in response to environmental cues with specific demands and considering the age and sex of the individual may prove to be a better biomarker for behavior and the development of behavior problems during adolescence.

Environmental cues which are considered by the adolescent as contexts in which social status could be gained but which also are experienced as stressful are likely to elicit a mixture of hormonal responses, as evidenced by positive coupling. Compounded with the developmental increases in activity of both axes, generally, adolescents may be more focused on status seeking milestones than in childhood, leading to more recruitment of both axes for regulation during stressful situations during adolescence relative to other developmental periods. Thus, we could hypothesize that coupling would remain positive in response to stressors among individuals who have life goals that include gaining social status, but that positive coupling may dissipate in adults who have more diverse goals.

Limitations and Future Directions

Although we were able to find significant within-person coupling in response to all three stressors, there was generally limited variability in the stress responses. Possibly as a result of this, coupling was positive, but also was overall a rather small effect. Thus, it remains to be seen whether hormone responses would be more or less coupled in response to more stressful environmental stimuli than what is experienced in a laboratory setting. However, although small, positive coupling appears to be a robust finding across several independent studies.

This study was limited because we did not examine estrogen. We chose not to examine estrogen because we were unable to find evidence that estrogen responds to stressors and the sampling strategy is very different. Additionally, while testosterone is present at measurable levels in girls, boys and pre-pubertal girls have very low levels of circulating estrogen, making reliable measurement of estrogen much more difficult, especially in saliva (Dorn et al., 2006; Shirtcliff et al., 2000). Nonetheless, it would be important to include estrogen in future studies.

Here, developmental effects were primarily assessed cross-sectionally rather than longitudinally. The second study (NIMH-NICHD) was longitudinal, but the sample size was somewhat small. Therefore, we are hesitant to conclude that there is no variability in coupling in response to venipuncture over time, as we may simply not have the power to detect such an effect. In the future, larger, longitudinal studies should be employed to replicate the effects shown here both within- and between- persons over time.

Despite these limitations, the present study showed consistently, across samples, contexts, and biological sampling procedures, that cortisol and DHEA and cortisol and testosterone were positively associated within-adolescents in response to acute stressors. Though testing associations with behavior was out of the scope of the current study, we highlight the importance of considering the effects of multiple hormones in order to better understand the biological underpinnings of behavior, especially during adolescence. It may be that during adolescence, in response to acute environmental stimuli, appraisal in acute challenges is likely dynamic and may be interpreted as an opportunity to gain social status or a threat to current social standing; some combination of stress, frustration, competition, and/or conflict. This appraisal may invoke activation of multiple hormones to respond effectively to stressors especially during adolescence in part because adolescence is a unique developmental transition particularly accompanied by hormonal changes and when social relationships are highly salient. Moving forward, self-report ratings for how stressful, frustrating, or distressing individuals found the task (or some other method of verifying the underpinnings for the task) will be important to include in order to glean better information about relations between subjective interpretations and physiological responses. This information may be especially valuable as future studies attempt to disentangle the components of a task that stimulates each hormone or the entire hormonal milieu. Our results clearly suggest the importance of considering multiple hormones, representing different axes, in a developmental framework. In the future, studies examining biological underpinnings of behavior and behavior problems may gain purchase by examining the hormonal milieu and changes in the hormonal milieu of adolescents rather than single hormones out of context.

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Table 1
Means and Standard Deviations of Saliva Hormone Levels in the AES Study (1)

	CDP						SPP					
	Boys			Girls			Boys			Girls		
	Mean	(SD)	(SD)	Mean	(SD)	(SD)	Mean	(SD)	(SD)	Mean	(SD)	(SD)
<u>Testosterone (pg/mL)</u>												
Collection 1	25.97	19.24	11.86	5.37	31.44	20.10	11.69	5.01				
Collection 2	24.48	19.51	10.25	5.58	31.62	22.67	12.00	5.13				
Collection 3	22.79	18.69	9.58	5.05	30.09	21.61	11.64	5.19				
<u>Cortisol (µg/dL)</u>												
Collection 1	0.18	0.10	0.16	0.07	0.18	0.09	0.17	0.08				
Collection 2	0.13	0.07	0.11	0.06	0.20	0.11	0.18	0.09				
Collection 3	0.11	0.06	0.09	0.05	0.14	0.07	0.16	0.08				
<u>DHEA (pg/mL)</u>												
Collection 1	63.79	28.19	83.44	43.08	60.08	26.98	75.64	35.96				
Collection 2	56.37	24.63	70.38	37.13	66.09	28.31	85.56	45.48				
Collection 3	51.95	23.84	65.97	33.03	64.16	26.30	84.78	44.25				

Note. Values are presented before windsorizing and log transformation. Hormones were collected via saliva. N = 106 boys, 107 girls. CDP = conflict discussion paradigm. SPP = social performance paradigm.

Table 2
Means and Standard Deviations of Serum Hormone Levels in the NIMH-NICHD Study (2)

	Venipuncture T1			Venipuncture T2			Venipuncture T3					
	Boys		Girls	Boys		Girls	Boys		Girls			
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)		
<u>Testosterone (ng/dL)</u>												
Collection 1	228.16	213.18	19.58	10.18	288.38	238.92	21.13	12.10	337.94	231.51	22.89	10.99
Collection 2	219.96	216.58	18.93	9.02	281.99	222.57	20.83	12.24	326.70	248.79	22.20	11.99
Collection 3	196.91	216.63	18.78	9.41	263.25	213.33	19.50	12.43	325.00	250.23	21.69	15.09
<u>Cortisol (µg/dL)</u>												
Collection 1	12.63	5.32	13.17	5.98	11.88	4.06	14.03	5.26	12.50	4.45	15.94	14.45
Collection 2	13.82	6.04	13.29	5.56	11.73	3.89	12.98	5.56	12.53	6.15	14.20	13.50
Collection 3	13.42	6.45	11.91	6.33	11.09	4.84	11.17	5.42	10.44	4.92	11.34	6.40
<u>DHEA (ng/dL)</u>												
Collection 1	230.13	133.01	231.13	147.86	233.24	124.05	299.42	204.08	282.44	153.85	316.72	199.90
Collection 2	271.59	162.57	258.36	185.16	258.88	135.04	301.99	206.32	297.22	155.03	285.02	155.75
Collection 3	255.75	165.01	244.98	206.51	247.49	120.38	264.12	207.75	264.47	142.84	264.29	175.09

Note. Values are presented before windsorizing and log transformation. Hormones were collected via blood. N = 56 boys, 52 girls.

Table 3
 Bivariate Coupling of Cortisol and DHEA Responsivity in Adolescents Experiencing Various Stress Paradigms

Parameter	Study 1			Study 2		
	CDP	SPP	Venipuncture	CDP	SPP	Venipuncture
	Estimate	SE	Estimate	SE	Estimate	SE
Fixed effects						
Intercept, γ_{00}	1.64*	.002	1.65*	.003	2.82*	.07
Coupling parameter, γ_{10}	.04*	.008	.03*	.008	.25*	.11
Effect of moderators on Cortisol Level						
DHEA Level, γ_{01}	.001	.002	.005*	.002	.18*	.04
Age, γ_{02}	-.001	.001	.004*	.001	.002	.004
Sex, γ_{03}	-.005*	.001	-.002	.002	-.006	.044
Age * Sex, γ_{04}	.001	.001	-.002	.001	-.003	.002
Effect of moderators on Coupling						
Age, γ_{11}	.009	.005	.02*	.005	-.004	.007
Sex, γ_{12}	-.005	.005	-.001	.005	.09	.07
Age * Sex, γ_{13}	-.004	.003	-.01*	.003	.002	.004
Random effects						
Level variance, u_{0j}	.0001*	<.0001	.0001*	<.0001	.03*	.007
Level variance across assessments, u_{2j}					.03*	.004
Coupling variance, u_{1j}	.0004*	.0001	.0001	.0001	.04*	.02
Coupling variance across assessments, u_{3j}					<.0001	<.0001
Residual, e_{ij}	.0001*	<.0001	.0001*	<.0001	.01*	.001
Fit statistics						
-2LL	-3872.1		-3683.9		-289.1	
AIC	-3864.1		-3675.9		-277.1	
BIC	-3850.7		-3662.5		-261.1	

Note. Coupling refers the within-person association of cortisol with DHEA. CDP = the conflict discussion paradigm. SPP = the social performance paradigm. $-2LL = \log$ likelihood function. AIC = Akaike information criterion. BIC = Bayesian information criterion. SE = standard error.

* = $p < .05$. N = 106 boys and 107 girls for the CDP and SPP (Study 1). N = 52 boys and 56 girls for the venipuncture (Study 2).

Table 4
 Bivariate Coupling of Cortisol and Testosterone Responsivity in Adolescents Experiencing Various Stress Paradigms

Parameter	Study 1			Study 2		
	CDP	SPP	Venipuncture	CDP	SPP	Venipuncture
	Estimate	SE	Estimate	SE	Estimate	SE
Fixed effects						
Intercept, γ_{00}	1.64*	.003	1.64*	.003	2.84*	.09
Coupling parameter, γ_{10}	.04*	.01	.04*	.01	-.25	.16
Effect of moderators on Cortisol Level						
Testosterone Level, γ_{01}	.005*	.002	.004*	.002	-.03	.03
Age, γ_{02}	-.004*	.002	.001	.002	.005	.005
Sex, γ_{03}	-.002	.002	.002	.002	-.01	.06
Age * Sex, γ_{04}	.002*	.001	-.001	.001	-.002	.003
Effect of moderators on Coupling						
Age, γ_{11}	-.02*	.008	.02†	.009	-.003	.009
Sex, γ_{12}	.002	.007	<.001	.009	.26*	.11
Age * Sex, γ_{13}	.009	.005	-.01†	.006	.003	.006
Random effects						
Level variance, u_{0j}	<.0001*	<.0001	.0001*	<.0001	.03*	.007
Level variance across assessments, u_{2j}					.03*	.004
Coupling variance, u_{1j}	.0008*	.0003	.0007*	.0003	.04*	.03
Coupling variance across assessments, u_{3j}					<.0001	<.0001
Residual, e_{ij}	.0001*	<.0001	.0001*	<.0001	.02*	.002
Fit statistics						
-2LL	-3810.7		-3634.7		-116.9	
AIC	-3802.7		-3626.7		-104.9	
BIC	-3789.3		-3613.3		-88.8	

Note. Coupling refers the within-person association of cortisol with DHEA. CDP = the conflict discussion paradigm. SPP = the social performance paradigm. $-2LL = \log$ likelihood function. AIC = Akaike information criterion. BIC = Bayesian information criterion. SE = standard error.

* = $p < .05$. N = 106 boys and 107 girls for the CDP and SPP (Study 1). N = 52 boys and 56 girls for the venipuncture (Study 2).

Table 5
Trivariate Coupling of Responsivity, Cortisol, DHEA and Testosterone in Adolescents Experiencing Various Stress Paradigms

Parameter	Study 1			Study 2		
	CDP	SPP	Venipuncture	CDP	SPP	Venipuncture
	Estimate	SE	Estimate	SE	Estimate	SE
Fixed effects						
Intercept, γ_{00}	1.64*	.003	1.64*	.003	2.97*	.11
DHEA Coupling parameter, γ_{10}	.03*	.008	.02*	.009	.29*	.13
Testosterone Coupling parameter, γ_{20}	.02	.01	.03*	.014	-.03	.13
Effect of moderators on Cortisol Level						
DHEA Level, γ_{01}	.001	.002	.004†	.002	.19*	.05
Testosterone Level, γ_{02}	.003	.002	.001	.002	-.07*	.03
Age, γ_{03}	-.003	.002	.003	.002	.008	.006
Sex, γ_{04}	-.004*	.002	-.001	.002	-.11	.08
Age* Sex, γ_{05}	.001	.001	-.002	.001	-.006	.003
Effect of moderators on DHEA Coupling						
Age, γ_{11}	.01*	.005	.02*	.006	-.006	.008
Sex, γ_{12}	-.007	.005	<.001	.005	.08	.08
Age* Sex, γ_{13}	-.008*	.003	-.008*	.003	.003	.005
Effect of moderators on Testosterone Coupling						
Age, γ_{21}	-.02*	.008	.003	.009	.003	.007
Sex, γ_{22}	.003	.008	-.005	.009	.12	.09
Age* Sex, γ_{23}	.01*	.005	-.004	.006	-.001	.005
Random effects						
Level variance, u_{0j}	.0001*	<.0001	.0001*	<.0001	.04*	.02
Level variance across assessments, u_{3j}					.07*	.007
DHEA Coupling variance, u_{1j}	.0003*	.0001	.0001	.0002	.03	.04

Parameter	Study 1			Study 2		
	CDP	SPP	Venipuncture	CDP	SPP	Venipuncture
DHEA Coupling variance across assessments, u_{4i}						
Testosterone Coupling variance, u_{2j}	.0010*	.0003	.0007*	.0003	.02	.05
Testosterone Coupling variance across assessments, u_{5j}					.02	.06
Residual, e_{it}	.00004*	<.0001	<.0001*	<.0001	.01*	.001
Fit statistics						
-2LL	-3906.8		-3682.4			-237.6
AIC	-3892.8		-3668.4			-211.6
BIC	-3869.3		-3644.9			-176.9

Note. Coupling refers the within-person association of cortisol with DHEA. CDP = the conflict discussion paradigm. SPP = the social performance paradigm. -2LL = log likelihood function. AIC = Akaike information criterion. BIC = Bayesian information criterion. SE = standard error.

* = $p < .05$. N = 106 boys and 107 girls for the CDP and SPP (Study 1). N = 52 boys and 56 girls for the venipuncture (Study 2).