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Patterns of Neuroendocrine Coupling in 9-Year-Old Children: Effects of Sex, Body-Mass Index, and Life Stress

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Abstract

Previous investigations have explored stress and pubertal hormones in parallel; it has been a recent development, however, to explore the relationships between different hormones during puberty, and how this hormonal cross-talk may be influenced by the environment. The current study investigated neuroendocrine coupling, or the extent to which hormones are correlated within the individual, and also investigated early life stressors that may influence coupling. Participants were 405 adrenarcheal children (mean Tanner stage = 1.73 for girls and 1.38 for boys) from a longitudinal study who provided saliva samples for analysis of cortisol, dehydroepiandrosterone (DHEA), and testosterone. Saliva was collected when children were 9-years-old, while early life stressors were assessed at each longitudinal assessment (ages 3, 6, and 9). Results from multi-level modeling (MLM) analyses provided evidence of positive cortisol-dehydroepiandrosterone (DHEA) and cortisol-testosterone coupling in middle childhood, and identified body mass index as a predictor of the strength of hormone coordination. While exposure to stressful life events did not impact cortisol-DHEA coupling patterns, stress interacted with sex to predict looser cortisol-testosterone coupling in girls, but not boys. The current study adds to the existing literature on the development of neuroendocrine coupling, and provided further evidence of sex differences in the impact of stress. Furthermore, hormone coupling may be investigated in the future as a mechanism by which puberty is associated with negative behavioral outcomes.

Keywords

neuroendocrine coupling; puberty; middle childhood; BMI; stress; cortisol; testosterone; DHEA

Puberty, the transition from childhood to adulthood, is not a singular event, but rather a series of hormonal, neural, psychological, and social changes taking place over many years. While all healthy adolescents experience these changes, there has been growing interest in

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individual differences that may alter or disrupt typical developmental processes during puberty. In large part, this has been motivated by research showing that the rates of many negative outcomes, including psychological disorders and serious risk-taking behaviors, rise during and closely after puberty (Downing & Bellis, 2009; Graber, 2003; Hyde, Mezulis, & Abramson, 2008).

The myriad events of puberty are primarily initiated through hormonal changes, many of which occur well before puberty is outwardly apparent, and occur as two separate but related processes. Adrenarche begins when the adrenal gland matures and releases dehydroepiandrosterone (DHEA), typically between the ages of 6 and 8 for girls and ages 7 and 9 for boys (Auchus & Rainey, 2004). DHEA, which is an adrenal androgen largely regulated by the HPA axis, plays a role in the physical maturation associated with puberty, as rising levels of the hormone are associated with the development of pubic hair, oily skin, and voice changes. While DHEA has an important role as a sex hormone during puberty (Azziz et al., 2004; Byrne et al., 2017), it also has significant effects on brain development, especially during adrenarche (Campbell, 2011). Specifically, DHEA has been shown to promote neurogenesis (Maninger, Wolkowitz, Reus, Epel, & Mellon, 2009) as well as play a neuroprotective role during periods when the brain is especially vulnerable to environmental insults (Campbell, 2011). Throughout the lifespan, DHEA also acts in concert with cortisol during times of stress (Hucklebridge, Hussain, Evans, & Clow, 2005), largely in a neuroprotective function (Dismukes et al., 2016).

The second phase of puberty occurs when the hypothalamic-pituitary-gonadal (HPG) axis initiates a cascade of hormonal changes, which ultimately lead to the release of sex hormones (testosterone and estrogen) from the gonads. These hormones are responsible for both secondary sex characteristics and neural maturation during adolescence; the release of sex hormones and subsequent physical changes are known as gonadarche (Rosenfield, Cooke, & Radovick, 2008; Saenger & Dimartino-Nardi, 2001; Styne, 2004). Gonadarche occurs later than adrenarche, beginning normatively as early as ages 9 or 10 for girls, and approximately 1 year later for boys (Grumbach & Styne, 2003).

Although most investigations of puberty have considered hormones in isolation, recent studies have highlighted the relationship *between* hormone levels across time, also known as neuroendocrine coupling (Marceau et al., 2015; Mehta & Prasad, 2015; Simmons et al., 2015; Turan, Tackett, Lechtreck, & Browning, 2015). Results from both cross-sectional and longitudinal studies in adult human and animal samples suggest that the HPG and HPA axes regulate one another, although the direction of this effect has been inconsistent (Chichinadze & Chichinadze, 2008; Hardy et al., 2005). Further exploration into these inconsistencies has revealed that while HPG axis hormones increase in response to acute stress (Lennartsson, Kushnir, Bergquist, Billig, & Jonsdottir, 2012; Phan et al., 2017), exposure to chronic stress may result in decreases in sex steroid hormones, such that HPG axis functioning is inhibited when higher concentrations of HPA hormones are present, and vice versa (Elias, 1981; Roy, Kirschbaum, & Steptoe, 2003; Viau, 2002; Zilioli & Watson, 2012). This arrangement is advantageous as steroid hormones, such as testosterone, can protect the organism against the deleterious effects of excessive cortisol during times of acute stress (including memory deficits, cognitive dysfunction, and deficits in processing of emotional information; Lupien,

Maheu, Tu, Fiocco, & Schramek, 2007; Prickaerts & Steckler, 2005). Conversely, during times of high chronic stress, gonadal functioning may be inhibited in favor of survival activities (Gomez, Manalo, & Dallman, 2004; Handa et al., 1994).

During childhood and adolescence, however, “positive” (i.e., higher levels of one hormone predicting higher levels of another hormone) or even null coupling between testosterone and cortisol may be developmentally advantageous, as it would be counterproductive for one hormone to inhibit another during times of growth and development (Shirtcliff & Ruttle, 2010). Research in pre-pubertal male rats found that testosterone levels are unaffected by HPA functioning and vice versa, which may be beneficial because biological resources are not being used to regulate reproductive functioning during times of stress (Gomez, Houshyar, & Dallman, 2002; Gomez, Manalo, & Dallman, 2004; Lürzel, Kaiser, Krüger, & Sachser, 2011; Romeo, Lee, Chhua, McPherson, & McEwen, 2004). In early and middle adolescence, however, the pattern may begin to change to favor positive cortisol-testosterone coupling (Dismukes et al., 2014; Johnson et al., 2014; Marceau et al., 2015; Turan, Tackett, Lechtreck, & Browning, 2015). For example, in a mixed-sex human sample (mean age = 13.7 years, range = 11–16 years), Marceau et al. (2015) found that cortisol and testosterone were overall positively coupled, although the magnitude of the coupling was stronger for female participants.

In contrast, DHEA-cortisol coupling is hypothesized to become more positive as adolescents transition into adulthood (Hucklebridge, Hussain, Evans, & Clow, 2005; Ruttle et al., 2015; Tung, Lee, Tsai, & Hsiao, 2004). For example, Oskis, Clow, Thorn, Loveday, and Hucklebridge (2012) found only a trend-level evidence of positive cortisol and DHEA coupling in a sample of 10–18 year olds. However, DHEA and cortisol are positively coupled in adults (Hucklebridge, Hussain, Evans, & Clow, 2005). While the relationship between DHEA and cortisol remains positive across the lifespan, looser coupling during childhood and adolescence may lead to greater adaptability of the hormone systems to react to pubertal changes and stressful events, which is especially important during such active periods of neural growth (Campbell, 2011).

There is growing evidence that environmental stress can impact the pubertal transition and related hormones (Belsky et al., 2007; Ellis & Garber, 2000; Graber, Brooks-Gunn, & Warren, 1995; Romans, Martin, Gendall, & Herbison, 2003; Saxbe & Repetti, 2009). Research suggests that early exposure to negative life events, including maltreatment, abuse, or neglect, is related to HPA axis dysregulation (Essex, Klein, Cho, & Kalin, 2002; Miller, Chen, & Zhou, 2007; Tarullo & Gunnar, 2006), with effects on both cortisol and DHEA (Ellis & Essex, 2007). With regard to hormone interactions, Simmons et al. (2015) found that observed maternal aggression significantly moderated cortisol’s influence on testosterone and DHEA’s influence on both cortisol and testosterone levels in both girls and boys (mean age = 15.56 years), although the nature of these relationships differed between the sexes. In girls, cortisol and testosterone, as well as cortisol and DHEA, were only positively associated in girls who had experienced low or average levels of maternal aggression three years prior to the hormone collection; girls who had experienced higher levels of maternal aggression demonstrated no relationship between the hormones. In boys, however, high levels of maternal aggression predicted positive correlations between cortisol

and testosterone, and cortisol and DHEA, while low levels of maternal aggression predicted a *negative* relationship between the two sets of hormones.

Ruttle et al. (2015) assessed stress in children prospectively between the ages of 1 month and 4.5 years old, and measured cortisol, DHEA, and testosterone levels at ages 11, 13, and 15 years. Overall, cortisol-DHEA coupling was positive at age 11, and became progressively more positive through age 15, while cortisol-testosterone coupling was positive at age 11, but became negative at ages 13 and 15. Exposure to stress played a significant role in hormone functioning, however. Similar to non-exposed children, children exposed to higher levels of early stress displayed positive cortisol-DHEA coupling at age 11, but by age 13 cortisol and DHEA were more tightly positively coupled in exposed children than non-exposed children; by age 15, cortisol-DHEA coupling had plateaued for the exposed children, while it continued to become more tightly positively coupled in non-exposed children.

While cortisol-DHEA coupling was impacted by stress in both sexes, a significant sex effect emerged when assessing the impact of early life stress on cortisol-testosterone coupling. Specifically, cortisol-testosterone coupling was not impacted by early life stress in boys at any age, but girls who were exposed to stress displayed more adult-like (negative) coupling earlier in development; however, this specific difference between exposed and non-exposed girls did not emerge until age 13. Prior to age 13, exposed girls demonstrated tighter positive coupling than non-exposed girls, who demonstrated positive coupling that was somewhat weaker in magnitude. These age effects are noteworthy especially when considering that gender differences in hormone pathways differ between childhood and adolescence. Specifically, while hormone pathways are similar for male and female children, adolescent male hormone development is marked by a rapid rise in testosterone, while adolescent female hormone development features a more gradual rise in testosterone and cyclic changes in other steroid hormones (Granger, Schwartz, Booth, & Arentz, 1999; Grumbach & Styne, 2003). Taken together, the two previous studies support the hypotheses that there are sex differences with regard to the impact of stress on neuroendocrine coupling, which may be further impacted by both age at stress exposure and developmental stage at hormone collection.

Neuroendocrine coupling has not been investigated in adrenarcheal children, which is notable considering some research pointing to adrenarche as a period of specific vulnerability for brain development and later negative outcomes (Byrne et al., 2017). Hence, the current study was undertaken to examine cortisol-DHEA and cortisol-testosterone coupling in a prospective community sample of 9-year-old children. We extended prior research by determining whether the same pattern of findings demonstrated in Ruttle et al. (2015) are present during adrenarche but prior to gonadarche. Extrapolating from Ruttle et al.'s findings, we tentatively hypothesized that both cortisol-DHEA and cortisol-testosterone coupling would be positive in our sample.

We also tested the relationship of pubertal development and body mass index (BMI) to hormone coupling. Although previous studies have examined the relationship between pubertal development and hormone coupling, the restricted age range of participants in the

current study allows us to test the effect of pubertal development while minimizing the confound of age. Furthermore, to our knowledge, no previous study has examined the relationship of BMI and coupling, which is noteworthy considering the well-established role of BMI in the initiation of pubertal changes (Kaplowitz, 2008). Therefore, we explored whether BMI impacted the strength of cortisol-DHEA and/or cortisol-testosterone coupling.

Finally, we examined the impact of stressful life events to determine if patterns of coupling were impacted by psychosocial factors. While Ruttle et al. (2015) reported an effect of early life stress on hormone coupling patterns over time, such that children exposed to early life stress demonstrated more adult-like coupling patterns earlier in development, these effects did not significantly impact cortisol-DHEA coupling patterns until age 13. However, Ruttle et al. studied only distal stressors, and did not include measures of stressors occurring during adrenarche but prior to gonadarche. Although early stress may have either a dampening or sensitizing effect on the impact of future stress, previous research suggests that children and adolescents are potentially more sensitive to stress occurring *during* the hormonal cascade that takes place during adrenarche, rather than prior to it; these findings are further supported by lab studies suggesting increased sensitivity to concurrent stress (Byrne et al., 2017; Romeo & McEwen, 2006). Therefore, we examined the impact of stress on patterns of hormone coupling in 9-year old children, with stressful life events measured both prior to and concurrent with the start of adrenarche. While Simmons et al. (2015) assessed stress during the pubertal transition, they measured stress retrospectively and measured pubertal hormones later in development (mean age = 14.91). In contrast, the current study assesses stress and hormones both prospectively and concurrently.

We hypothesized that children who experience higher levels of life stress prior to adrenarche would demonstrate a similar magnitude of positive cortisol-DHEA coupling as those children who do not experience these stressors; higher levels of stress *during* adrenarche, however, should be associated with stronger positive cortisol-DHEA coupling. Cortisol and testosterone would be more positively coupled in those children exposed to high levels of either prior or concurrent life stress than in non-exposed children, consistent with Ruttle et al.'s findings. We also explored whether this effect varied by sex, considering both theory (Martel, 2013) and empirical evidence that females are more susceptible to the negative impacts of stress, especially around puberty (Hankin, Mermelstein, & Roesch, 2007; Keenan & Hipwell, 2005; Kochanska, 1997; Taylor, 2006; see Bale & Epperson, 2015 for a review), and that the greater susceptibility in females can influence hormone coupling patterns (Ruttle et al., 2015).¹

Method

Participants

Participants were 405 9-year-old children from a larger longitudinal study (Olino et al., 2010). The initial sample was recruited through commercial mailing lists of families with a 3-year-old child living with at least one biological parent within a 20-mile radius of the

¹Notably, DHEA-testosterone coupling was not examined in the current analyses due to our focus on dual-axis cross-talk between the HPG and HPA axes.

university; this list was culled by an outside agency from a variety of public and commercial sources. Only 1 child per family was included in the study. 559 children participated in the initial assessment ($M = 3.5$ years, $SD = .26$). An additional 50 minority families were recruited to increase the diversity of the sample in the next wave of assessments, bringing the total sample to 609 children. Of this total sample, 512 children (462 of the original 559 participants, and the additional 50 children recruited for the second wave) completed assessments when they were approximately 6 years old ($M = 6.08$ years, $SD = .41$). Of the 609 children, 490 children completed a third wave of assessments at age 9. The current sample was drawn from the families who completed saliva collection at age 9 ($N=419$; 85.5% of age 9 sample). After applying exclusion criteria (see below), the final sample consisted of $N = 405$ participants who were 9.28 years old ($SD = .41$) at the time of assessment. Participants were 53.1% male, and primarily Caucasian (89.6%; 7.4% African American, 2.7% Asian, 0.2% Native American) and non-Hispanic/Latino (88.6%; 11.4% Hispanic/Latino). Based on the Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988) and using Shirtcliff, Dahl, & Pollak's (2009) conversion to the Tanner metric, youth were on average, at Tanner stage 1.73 ($SD=.61$) for girls and 1.38 ($SD=.45$) for boys, suggesting that the majority of the current sample was in the earliest stages of visible pubertal development. 56.3% of the participants' mothers and 43.7% of their fathers had completed a 4-year college degree. Regarding psychiatric diagnoses in this community sample, lifetime anxiety disorders were the most prevalent (21% of the sample, although the majority of these children were diagnosed with specific phobias), followed by elimination disorders (14%), and attention-deficit/hyperactivity disorder (ADHD; 13%).

Children who completed saliva collection at the age 9 assessment but were not included in the final sample due to exclusion criteria ($N = 14$) did not differ from included children with regard to age, sex, race/ethnicity, parental education, and pubertal development. Of the full 609 children in the study, children who were and were not included in the present analyses did not differ on sex, race/ethnicity, or parental education.

Procedure

Information about stressful life events was collected at three waves (when children were approximately 3, 6, and 9 years old), and children's saliva and height and weight were collected at the age 9 assessment. Parents were instructed how to conduct the at-home saliva collection during their in-person study visit, and were subsequently contacted by study staff to answer questions and coordinate sample pick-up.

Measures

Hormone collection and assay—Children's saliva was collected via passive drool 30 minutes after waking on three consecutive weekdays, resulting in three saliva samples per participant from which cortisol, DHEA, and testosterone were assayed. Samples were collected in this way because peak hormone concentrations in saliva are typically observed within the first waking hour of the day, with peak cortisol observed approximately 30 minutes after awakening (Edwards, Clow, Evans, & Hucklebridge, 2001; Stadler et al., 2016). Participants were instructed to freeze saliva samples immediately after collection until a member of the study staff retrieved the samples from the participants' homes. The

samples were then stored at -20°C until they were transported on dry ice to the Biochemistry Laboratory at the University of Trier in Trier, Germany for assay. All samples were assayed for DHEA and testosterone using commercially available enzyme immunoassays specifically designed for use with saliva using the manufacturer's recommended protocol (Salimetrics, State College, PA). Cortisol was assayed using a time-resolved fluorescence immunoassay with fluorometric end-point detection (DELFI). The intra-assay coefficient of variation for cortisol was 5.4, while the inter-assay coefficient of variation was 9.8. From the same aliquot and at the same lab, all samples were assayed for DHEA and testosterone using commercially available enzyme immunoassays specifically designed for use with saliva using the manufacturer's recommended protocol (Salimetrics, State College, PA). The intra-assay coefficients of variation for DHEA and testosterone were 2.5 and 6.7, respectively, and the inter-assay coefficients of variation were 7.1 (DHEA) and 9.0 (testosterone). All samples were assayed in duplicate and then the average of the duplicates was natural log transformed to reduce skew. All analyses used transformed cortisol, DHEA and testosterone values. For multi-level modeling analyses, coordination of hormone values across the three days was examined, so each day's hormone samples were investigated separately.

Sample exclusion—Several criteria were used to determine sample exclusion. First, samples that were not frozen in a home freezer or that melted in transit to the university were excluded, as sample accuracy may be compromised following a freeze-thaw cycle. Along with saliva samples, participants returned a diary in which they recorded information about the time samples were taken. Any DHEA or testosterone sample that was collected more than 30 minutes after the intended time was excluded; similarly, any cortisol sample that was collected more than 15 minutes before or after the intended time was excluded. As all three hormones have some diurnal pattern, including samples that were taken more than 30 (DHEA and testosterone) or 15 (cortisol) minutes after their intended time may obscure the results, as we want to compare hormone levels around the same peak time. The timing exclusion criterion for cortisol samples was more stringent due to cortisol's sharper diurnal peak compared to DHEA or testosterone (Matchock, Dorn, & Susman, 2007). Additionally, samples were excluded if the child was using an oral or inhaled corticosteroid medication, an antipsychotic, or methylphenidate – extended release (Concerta) during sample collection, as these medications have been shown to affect hormone levels in children and adolescents (Granger et al., 2012).

Following sample exclusion, children were included in the multi-level modeling analyses if they had at least one complete day of valid cortisol, DHEA, and testosterone samples to allow for observation of correlations between hormones. 419 children completed home saliva collection at age 9; of these children, 405 had sufficient hormone data to be included in the MLM analyses.

Pubertal development—The Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988) was administered to parents at the age 9 assessment. The PDS assesses pubertal development using five items. Items for both boys and girls include growth of body hair, skin changes (especially pimples), and growth in height; items for boys only

include voice deepening and growth of facial hair; items for girls only include breast development and menstruation. Each item is rated on a scale from 1 (not yet started) to 4 (seems complete). For the purposes of this study, only maternal reports of pubertal development were used in light of research suggesting that mothers are accurate reporters of their children's pubertal development, especially early in development (Coleman & Coleman, 2002). For ease of comparison with other investigations of pubertal development, Shirtcliff, Dahl, & Pollak's (2009) conversion of PDS score to the Tanner staging metric was used in the current analyses.

Body mass index (BMI)—We measured participants' height and weight at the age 9 assessment to calculate BMI (Keys et al., 1972). Study staff were trained in appropriate anthropometric measurement techniques, including instructing participants to stand up straight and remove shoes and excess clothing prior to being weighed.

Stressful life events—At ages 3 and 6, information about stressful life events was collected from parents during the Preschool Age Psychiatric Assessment (PAPA; Egger & Angold, 2004) interview; information about the same stressful life events was collected from parents and children at the age 9 assessment during the Kiddie Schedule for Affective Disorders (K-SADS-PL; Kaufman et al., 1997) interview. Stressful life events from the past three years were assessed at each study visit. Stressful life events included major transitions (e.g., moving to a new house, birth of a new sibling or introduction of a new child into the home, being removed from the family home), chronic stressors (e.g., financial distress, neglect), and traumas (e.g., natural disasters, exposure to domestic violence, physical/sexual abuse). At each assessment, stressful events were counted as “present” or “absent,” and the final stress score was a sum of all endorsed stressors in the given time period (e.g., prior to age 3, between ages 3 and 6, or between ages 6 and 9). For participants in the current sample, assessment of stressful life events was completed for 374 children at age 3 ($M = 3.40$, $SD = 2.39$; range = 0–14), 392 children at age 6 ($M = 3.77$, $SD = 2.38$; range = 0–17), and 405 children at age 9 ($M = 2.12$, $SD = 1.66$; range = 0–12).

Data analysis

Data were analyzed using multi-level modeling (MLM) procedures. MLM is useful for partitioning variance when data are “nested” within grouped units (e.g., multiple points within individuals). For the current study, level 1 variables included cortisol, DHEA, and testosterone values from three different days, while level 2 variables were those that are constant within the individual at a given time point (e.g., sex, pubertal development, BMI, and stress). This enabled us to test for evidence of neuroendocrine coupling, as well as to examine predictors associated with different patterns of hormone coupling.

First, we tested unconditional means models to determine whether the distribution of variance across levels 1 and 2 was sufficient to justifying the use of MLM, and the placement of consequent random effects terms.

The mixed unconditional means model for cortisol was as follows:

$$CORTISOL_{ti} = \beta_{00} + r_{0i} + e_{ti}$$

where β_{00} is a given individual's mean cortisol level, r_{0i} is the random effect at level 2 (indicating between-person individual differences in cortisol levels), and e_{ti} represents individual error (within-person variance across cortisol measurement). The level 1 intraclass correlation (ICC) for cortisol was .48; using a threshold of .25 (Guo, 2005; Heinrich & Lynn, 2001), this indicated sufficient distribution of variability across levels to warrant the use of MLM. We also tested the mixed unconditional means models for DHEA and testosterone, as those hormones would be used as level 1 independent variables:

$$DHEA_LN_{ti} = \beta_{00} + r_{0i} + e_{ti}$$

$$TESTO_LN_{ti} = \beta_{00} + r_{0i} + e_{ti}$$

The ICCs for DHEA and testosterone were .77 and .68, respectively, also meeting the minimum threshold criteria, and suggesting that a random effect should be included at Level 2 for both predictors.

We then examined models with cortisol as the level 1 outcome variable, and DHEA or testosterone as level 1 predictors; cortisol-DHEA and cortisol-testosterone coupling served as the primary outcomes of interest.

$$CORTISOL_{ti} = \beta_{0i} + \beta_{1i} * (DHEA_LN_{ti}) + e_{ti}$$

$$CORTISOL_{ti} = \beta_{0i} + \beta_{1i} * (TESTO_LN_{ti}) + e_{ti}$$

In these models, $CORTISOL_{ti}$ is the given individual's cortisol level at time 1, β_{0i} is the predicted level of cortisol at the mean level of DHEA/testosterone, β_{1i} is the coefficient describing the concurrent changes in cortisol and DHEA/testosterone across days, and e_{ti} represents individual error. Effect size was calculated by dividing the difference of the null and random variance by the null variance (Woltman, Feldstain, MacKay, & Rocchi, 2012):

$$r^2 = \frac{\sigma^2_{\text{null}} - \sigma^2_{\text{random}}}{\sigma^2_{\text{null}}}$$

We also investigated a model with DHEA and testosterone simultaneously entered as level 1 predictors, in order to ascertain whether the strength of the relationship between cortisol and testosterone would be diminished when considering DHEA as well (as testosterone in this age range is primarily adrenal in origin).

$$CORTISOL_{ij} = \beta_{0j} + \beta_{1j} * (DHEA_LN_{ij}) + \beta_{2j} * (TESTO_LN_{ij}) + r_{ij}$$

The level 2 variables of interest were included in separate models. The first and second set of models included BMI and PDS scores as level 2 variables predicting cortisol-DHEA and cortisol-testosterone coupling, in order to determine the relationships between body growth, pubertal development, and coupling patterns. As our participants were of a restricted age range, any investigation of how differences in pubertal development and body growth may impact hormone coupling were minimally confounded by chronological age. An example of these level 2 models is shown below: Level 1 model: $CORTISOL_{ij} = \beta_{0j} + \beta_{1j} * (DHEA_LN_{ij}) + r_{ij}$

Level 2 model:

$$\beta_{0j} = \gamma_{00} + \gamma_{01} * (CHILD_BMI_j) + u_{0j}$$

$$\beta_{1j} = \gamma_{10} + \gamma_{11} * (CHILD_BMI_j) + u_{1j}$$

where γ_{00} is the intercept term predicting level of cortisol from the individual's DHEA level when the between-person predictor (BMI) is at the sample mean level, γ_{01} is the coupling parameter at the sample mean BMI, and γ_{11} is the coefficient describing the extent to which BMI influences cortisol-DHEA coupling.

The second set of models included stressful life events from different age ranges on level 2; the number of stressful life events in a given time period (e.g., age 3, 6, or 9) were entered as level 2 variables in the stress model. Summed stress scores were computed separately for different age ranges in order to determine whether stress at various developmental points may differentially impact neuroendocrine coupling, and the three summed scores were entered into the same model. Interactions with sex were also examined in light of previous research suggesting that sex may moderate the association between stress and hormone coupling.

Results

Table 1 shows bivariate correlations between cortisol, DHEA and testosterone levels, averaged across days, and the variables of interest (sex, pubertal development, and BMI) described above. Briefly, all three hormones were positively correlated (r s from .27-.63, all p s < .001), and BMI and PDS were significantly positively associated with DHEA and testosterone, but not cortisol. In addition, in this sample of 9-year-olds, girls had higher testosterone levels than boys. Finally, there were positive correlations between testosterone and stressful life events at age 9 ($r = .11$, $p < .05$), and DHEA and stressful life events at age 6 ($r = .11$, $p < .05$).

Multilevel models testing within-individual coupling between hormones

In models investigating hormone coupling, we found that both DHEA and testosterone were significantly positively coupled with cortisol, indicating that levels of these hormones rise and fall concurrently (DHEA: $\beta_{10} = .23$, $SE = .06$, $p < .001$; testosterone: $\beta_{10} = .79$, $SE = .11$, $p < .001$). The effect sizes indicate that coupling is greater for cortisol-testosterone (level 1 $r^2 = .54$) than for cortisol-DHEA (level 1 $r^2 = .37$). Furthermore, entering both DHEA and testosterone as level 1 predictors reduced cortisol-DHEA coupling to nonsignificance ($\beta_{10} = .02$, $SE = .04$, $n.s.$), but the significance of cortisol-testosterone coupling was maintained ($\beta_{20} = .73$, $SE = .11$, $p < .001$).

Multilevel models testing predictors of coupling between hormones

PDS—We first entered maternal report of child's PDS score as a level 2 predictor to ascertain the impact of pubertal development on hormone coupling patterns. In all analyses, cortisol was used as the outcome variable, DHEA or testosterone was entered as a level 1 predictor, and PDS was entered as a level 2 variable modeled on both the cortisol intercept and the DHEA/testosterone slope. In models using PDS as a level 2 predictor, no significant effects emerged for either cortisol-DHEA or cortisol-testosterone coupling (Table 2).

BMI—We then examined whether body size was related to coupling patterns. BMI was significantly associated with decreased cortisol levels, $\gamma_{01} = -.02$ ($SE = .01$), $p < .05$, as well as tighter cortisol-DHEA ($\gamma_{11} = .04$ [$SE = .02$], $p < .001$) and cortisol-testosterone ($\gamma_{11} = .05$ [$SE = .02$], $p < .05$) coupling patterns (Table 3). Further investigation of this link revealed that DHEA and testosterone levels were higher in children who were overweight/obese (based on Center for Disease Control guidelines; CDC, 2009), rather than simply of above average weight.

Child stress—Next, mixed models were run modeling child life stress at three time points (ages 3, 6, and 9) on both the cortisol intercept and DHEA/testosterone slopes. No significant main effect associations emerged in these analyses; there was, however, a significant sex by stress interaction. Specifically, stress at the age 6 assessment interacted with sex to predict changes in cortisol-testosterone coupling, $\gamma_{13} = .23$ ($SE = .12$), $p < .05$ (Table 4). Decomposing this interaction revealed that stress significantly impacted coupling patterns in girls ($\gamma_{12} = -.41$ [$SE = .20$], $p < .05$), but did not affect boys' patterns of cortisol-testosterone coupling ($\gamma_{12} = .05$ [$SE = .07$], $n.s.$). Further probing revealed that girls who were exposed to higher levels of stress demonstrated looser cortisol-testosterone coupling than those girls exposed to lower levels of stress (see Figure 1).²

Discussion

The current study utilized a prospective, longitudinal design to investigate hormone coupling in a sample of 9-year-old children, while also investigating the impact of BMI and stress during multiple developmental periods in influencing hormone coupling patterns. The study

²Notably, due to the strong relationship between BMI and hormone coupling, BMI was included in models testing the impact of stress on coupling; including this variable did not significantly impact our results, however.

had two primary hypotheses: 1) both cortisol-DHEA and cortisol-testosterone coupling would be positively associated in the sample as a whole; 2) life stress would not influence the magnitude of cortisol-DHEA coupling at age 9; in contrast, testosterone would be more positively coupled with cortisol in those children exposed to high levels of life stress than in non-exposed children. Our results largely confirmed these hypotheses, finding that both cortisol-DHEA and cortisol-testosterone coupling were positive in our sample of 9-year-olds, and that stress impacted cortisol-testosterone coupling, although the direction of this effect was reversed.

Coupling

Children in our sample demonstrated significant positive coupling between both cortisol-DHEA and cortisol-testosterone, an effect which extends previous work in this area by demonstrating hormone coordination earlier in development than previously documented. Our coupling patterns are consistent with Ruttle et al. (2015), in that their participants demonstrated consistently positive cortisol-DHEA coupling which became tighter across time, and their participants' cortisol-testosterone coupling was positively coupled at the earliest age they examined (age 11) and then became increasingly negatively coupled across time. Marceau et al. (2015) similarly found positive cortisol-DHEA and cortisol-testosterone coupling in their sample of 11–16 year olds. When we controlled for testosterone, however, the cortisol-DHEA relationship was no longer significant. This suggests that in our 9-year-old sample, DHEA and testosterone have common adrenal origins, and the effect of DHEA on cortisol may have been overshadowed by the cortisol-testosterone coupling.

The coupling patterns we observed were not moderated by pubertal status, an outcome that is consistent with previous research (Marceau et al., 2014; Turan et al., 2015). Coupling was moderated by body size, in that children with higher BMI demonstrate tighter hormone coupling than those children with lower BMI; this pattern was strongest for cortisol-DHEA coupling. Relatedly, consistent with previous research in this area (Corvalán, Uauy, & Mericq, 2013; Genazzani, Pintor, & Corda, 1978; l'Allemand et al., 2002; McCartney et al., 2007), DHEA and testosterone levels were higher in children who were overweight or obese, rather than simply of above average weight. BMI may influence hormone coupling through multiple mechanisms including the possibility that greater adiposity is linked with slower movement of steroids in adipose tissue as well as proximal conversion of hormones into metabolic downstream hormones which impact on children's physiology. Further research, however, will be required to both replicate and clarify this result.

Coupling and life stress

Following previous literature in this area, we hypothesized that children exposed to greater life stress would demonstrate more adult-like hormone coupling patterns than those children exposed to lower levels of stress. Previous work has demonstrated that life stressors are related to both earlier adult-like hormone coupling, but also incomplete development of the coupling process; in other words, the hormones of children exposed to stress coordinate with one another *earlier* in development, but do not seem to *fully* coordinate as intended later in development. Although we are not able to trace the progression of coupling in our sample, we did find that exposure to stressful life events between ages 3 and 6 influenced cortisol-

testosterone coupling patterns in 9-year-old girls, but not boys. This finding was contrary to both our original hypothesis (that cortisol-testosterone coupling would be more positively coupled in exposed children than non-exposed children). Less positive coupling in exposed girls, however, may signal an early start to the transition to negative hormone coupling (which Ruttle et al. [2015] reported in their 13 and 15 year old girls). This may be a particularly salient time because 9-year old children are in the midst of adrenarche, when DHEA levels have been increasing for several years (Sumner et al., 1999), and, for some girls, in the very earliest parts of gonadarche, when gonadal steroid release is initiated. It is possible that stress during this time period influences subsequent coupling in both boys and girls, but only once adrenarche has been in progress for some time (as the girls in our sample were further into puberty than the boys). There is also evidence for sex differences in androgen receptors in the brain (Raznahan et al., 2010; Tsai, Taniguchi, Samoza, & Ridder, 2015), which could lead to dissimilar endocrine reactions to stress exposure in males versus females. Following the current sample through the pubertal transition will clarify whether this effect is due to sex or earlier maturation in girls.

Investigating hormone coupling patterns over time may further our understanding of the biological processes involved in the emergence of psychiatric illness in adolescents. Specifically, future research should examine psychiatric outcomes associated with dysregulated neuroendocrine coupling, which to date have not been addressed in either adult or adolescent samples. If early adolescent cortisol-DHEA coupling, for example, is more tightly positively coupled at an earlier age, but then fails to develop to its full capacity, DHEA may not be recruited to counter-balance the effect of cortisol in adulthood (Goodyer, Park, Netherton, & Herbert, 2001). This early but incomplete development of coupling patterns may have behavioral consequences both in adolescence and adulthood.

Strengths and limitations

The current study had a number of strengths, especially its examination of coupling and influences on coupling in a 9-year-old sample. While previous research has examined coupling in older samples, few studies have explored the relationships between hormones at the beginning of the pubertal transition. Other strengths include the use of a large community sample of children who have been followed prospectively for 6 years and longitudinal assessment of multiple types of environmental stressors. However, the current study also had several limitations. First, we did not assess estradiol or progesterone, which limits our understanding of dual-axis functioning in childhood, particularly within females. That many findings were specific to girls suggests this limitation is not profound, but it is possible that coupling with estradiol or progesterone might be even stronger than that observed with testosterone and DHEA. Second, the sample was primarily Caucasian and middle class, which may limit the generalizability of the findings. Finally, child psychiatric diagnoses were not considered in the current analyses; although beyond the scope of the current investigation, this will be an important variable to consider in future studies of hormone coupling.

The current study nevertheless contributes to the small literature on neuroendocrine coupling by examining patterns of *typical* neuroendocrine coupling in adrenarcheal children. Such

knowledge is essential to our understanding of both the normative changes that occur during puberty, and the relationship between stress and pubertal development. Furthermore, the current research raises important questions to be addressed in further research. Future work should follow adolescents longitudinally to understand how coupling patterns change throughout development, which factors may influence these patterns, and the implications of individual differences in coupling for later development and functioning.

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Highlights

- Positive hormone coupling is evident in adrenarcheal children.
- Body mass index strengthens the magnitude of these hormone relationships.
- Stressful life events alter hormone coupling patterns in girls, but not boys.

Figure 1.

High versus low stress between the ages of 3 and 6 is associated with looser cortisol-testosterone coupling in girls (left), but does not significantly impact cortisol-testosterone coupling in boys (right). Note: “High” and “Low” stress are defined as the average stress score plus/minus the standard deviation.

Table 1

Bivariate correlations among hormone levels and covariates

	Cortisol	DHEA	Testosterone	Sex	PDS	Race	BMI
Cortisol	–	.27***	.41***	.04	–.05	.05	–.06
DHEA		–	.63***	–.07	.19***	.06	.18***
Testosterone			–	–.16**	.26***	.06	.21***
Sex				–	–.32***	.06	–.03
PDS					–	.07	.29***
Race						–	–.05
BMI							–
Mean, SD/	2.11	4.09	3.26	195	7.06	1.73	18.26
N, %	.61	1.19	.46	46.5 ^f		90 ^w	3.53

* $p < .05$,** $p < .01$,*** $p < .001$ *Note.*^f = percent female participants;^w = percent white participants

Table 2

Multilevel models investigating the effect of PDS on hormone coupling

Parameter	DHEA		Testosterone	
	Estimate	SE	Estimate	SE
Fixed Effects				
Intercept, γ_{00}	2.07***	.03	2.07***	.03
Coupling parameter, γ_{10}	.23***	.06	.81***	.12
Effect of moderators on cortisol level				
PDS, γ_{01}	-.09	.07	-.09	.07
BMI, γ_{02}	-.01	.01	-.01	.01
Effect of moderators on coupling				
PDS, γ_{11}	.07	.11	-.20	.27
BMI, γ_{12}	.03	.02	.06	.03
Random effects				
Cortisol level variance, u_0	.27***	.52	.29***	.54
Coupling level variance, u_1	.28***	.53	1.02***	1.01
Residual, r	.17	.41	.13	.36

*
 $p < .05$,**
 $p < .01$,***
 $p < .001$

Table 3

Multilevel models investigating BMI as a predictor of hormone coupling

Parameter	DHEA		Testosterone	
	Estimate	SE	Estimate	SE
Fixed Effects				
Intercept, γ_{00}	2.07***	.03	2.07***	.03
Coupling parameter, γ_{10}	.23***	.05	.78***	.11
Effect of moderators on cortisol level				
BMI, γ_{01}	-.02	.01	-.02*	.01
Effect of moderators on coupling				
BMI, γ_{11}	.05***	.01	.06*	.03
Random effects				
Cortisol level variance, u_0	.27***	.52	.30***	.54
Coupling level variance, u_1	.29***	.54	1.05***	1.02
Residual, r	.17	.41	.13	.36

*
 $p < .05$,**
 $p < .01$,***
 $p < .001$

Table 4

Multilevel models investigating interactions between sex and age 6 stress as a predictor of cortisol-testosterone coupling

Parameter	Testosterone	
	Estimate	SE
Fixed Effects		
Intercept, γ_{00}	2.07 ***	.06
Coupling parameter, γ_{10}	.79 ***	.19
Effect of moderators on cortisol level		
Sex, γ_{01}	.01	.07
Age 6 stress, γ_{02}	-.01	.05
Sex $\times$ Age 6 stress interaction	.004	.03
Effect of moderators on coupling		
Sex, γ_{11}	.14	.24
Age 6 stress, γ_{12}	-.41 *	.20
Sex $\times$ Age 6 stress interaction	.23 *	.12
Random effects		
Cortisol level variance, u_0	.31 ***	.51
Coupling level variance, u_1	1.05 ***	1.02
Residual, r	.14	.37

*
 $p < .05$,

**
 $p < .01$,

 $p < .001$