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Neuroendocrine Coupling across Adolescence and the Longitudinal Influence of Early Life Stress

Paula L. Ruttle¹, Elizabeth A. Shirtcliff², Jeffrey M. Armstrong¹, Marjorie H. Klein¹, and Marilyn J. Essex¹

¹Department of Psychiatry, School of Medicine and Public Health, University of Wisconsin-Madison, 6001 Research Park Boulevard, Madison, WI 53719-1176

²Department of Psychology, University of New Orleans

Abstract

Drawing on conceptual models illustrating the advantages of a multisystemic, interactive, developmental approach to understanding development, the present study examines the covariation of stress and sex hormones across the adolescent transition and the effect of early life stress (ELS) on neuroendocrine coupling to gain insight into atypical development. Morning levels of cortisol, testosterone, and dehydroepiandrosterone (DHEA) were assessed at ages 11, 13, and 15; ELS was assessed during the infancy and preschool periods. Hierarchical linear modeling revealed that cortisol-DHEA coupling patterns progressed to tight, positive coupling across adolescence. Cortisol-testosterone coupling was positive at age 11 but became more negative at ages 13 and 15. Exposure to ELS resulted in more adultlike neuroendocrine coupling patterns earlier in life than non-exposed youth; however the effect of ELS on cortisoltestosterone coupling was unique to girls. Results illustrate trajectories of neuroendocrine coupling that may be unique to adolescence. Moderation by ELS suggests that early stress exposure may prompt earlier adultlike neuroendocrine coupling, particularly within girls, which may contribute to early pubertal development.

Keywords

cortisol; DHEA; testosterone; HPA-HPG coupling; early life stress; adolescence; hierarchical linear modeling

Introduction

Adolescence is a period characterized by unique physiological changes and developmental challenges. During adolescence, both the hypothalamic-pituitary-adrenal (HPA) and the hypothalamic-pituitary-gonadal (HPG) axes experience unprecedented maturation, including normative increases in hormones of both adrenal (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Shirtcliff et al., 2012) and gonadal (Grumbach, 2002) origin. A small handful of studies have identified HPA-HPG functioning in adults, with gonadal hormones typically

suppressed by the HPA axis and vice versa (Bingaman, Magnuson, Gray, & Handa, 1994; Elias, 1981; Handa et al., 1994). However, a unique pattern of neuroendocrine coupling may be present in adolescents due to maturational changes and accompanying rise in levels of various hormones of both axes (Marceau, Dorn, & Susman, 2012). The extent and direction of HPA-HPG hormonal coupling across adolescence have yet to be described, but prior research suggests this may be an important transitional period. Furthermore, exposure to early life stress (ELS) independently influences both the HPA and HPG axes (Essex, Shirtcliff, et al., 2011; Shirtcliff & Ruttle, 2010), which have been linked to a variety of negative outcomes directly or indirectly associated with adolescence, including advanced pubertal maturation (Ellis, Shirtcliff, Boyce, Deardorff, & Essex, 2011) and elevated rates of mental health problems (e.g., Essex, Shirtcliff, et al., 2011; Goodyer, Herbert, & Tamplin, 2003; Goodyer, Herbert, Tamplin, & Altham, 2000; Popma et al., 2007; Scerbo & Kolko, 1994; Shirtcliff, Zahn-Waxler, Klimes-Dougan, & Slattery, 2007). Understanding normative patterns of adrenal and gonadal hormone coupling from early to middle adolescence and identifying early risk factors for altered hormone coupling may help elucidate a mechanism through which ELS is translated into early sexual maturation and other negative adolescent outcomes.

One of the hallmark physiological changes associated with adolescence is the activation and maturation of the HPG axis. The axis is organized in prenatal and early postnatal development, and then experiences a period of relative quiescence during childhood (Romeo, 2003). The HPG axis is then reactivated with the commencement of pubertal onset (Ojeda & Terasawa, 2002), when a rise in the level of gonadotropin-releasing hormone from the hypothalamus produces an increase in the levels of leutinizing hormone and follicle-stimulating hormone from the pituitary. These hormones stimulate the gonads, resulting in increases in levels of sex hormones (Grumbach & Styne, 1998) that travel throughout the entire body—including directly crossing the blood-brain barrier—to promote the development of secondary sex characteristics and influence neural maturation (Sisk & Foster, 2004). Testosterone is the primary androgen associated with the HPG axis and is one of the key sex hormones responsible for advancing puberty. The rise in testosterone is responsible for the development of numerous secondary sex characteristics in boys (Biro, Lucky, Huster, & Morrison, 1995). Girls also demonstrate an increase in testosterone during puberty; although much smaller than the increase in boys (Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004; Legro, Lin, Demers, & Lloyd, 2000, there is some suggestion that very small testosterone changes in girls can be especially potent physiologically (Bateup, Booth, Shirtcliff, & Granger, 2002; van Honk et al., 2004; van Honk et al., 2001; Wirth & Schultheiss, 2007).

The HPA axis demonstrates a similar pattern of quiescence in childhood and re-activation and heightened activity during adolescence (Dahl & Gunnar, 2009; Gunnar et al., 2009), potentially driven by puberty directly (Matchock, Dorn, & Susman, 2007; Shirtcliff, Granger, Booth, & Johnson, 2005; Susman, Dorn, Inoff-Germaine, Nottelman, & Chrousos, 1997; Walker, Walder, & Reynolds, 2001) or indirectly (Essex, Armstrong, Burk, Goldsmith, & Boyce, 2011; Shirtcliff et al., 2012). HPA-axis activation results in the release of several hormones including the adrenal steroid, cortisol. Cortisol is one of the main hormonal end-products of the HPA axis that aids in mobilizing resources to help the body filter, encode and

enhance salient signals from the environment, including stressful contexts (Del Giudice, Ellis, & Shirtcliff, 2011). In the absence of a stressor, basal levels of cortisol typically follow a diurnal rhythm characterized by elevated morning levels and a gradual decline over the course of the day (Kirschbaum & Hellhammer, 1994; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001).

Dehydroepiandrosterone (DHEA), an endogenous hormone primarily derived from the HPA axis (for a review, see Krobath, Salek, Pittenger, Fabian, & Frye, 1999), is simultaneously released with cortisol, demonstrating a diurnal rhythm as well as increases alongside cortisol as part of the physiological stress response. While the majority of DHEA is produced by the adrenal cortex and regulated by the HPA axis, a small percentage of DHEA is produced by the gonads in males (Krobath et al., 1999). Furthermore, similar to testosterone, DHEA plays a role in advancing certain aspects of physical maturation in boys and girls (Dorn, Dahl, Woodward, & Biro, 2006; Shirtcliff, 2009). More specifically, at approximately 6 years of age, rising DHEA marks the commencement of adrenarche, i.e., the maturation of the adrenal gland (Auchus & Rainey, 2004), which eventually initiates the development of secondary sex characteristics such as pubic hair growth, acne, a small peripubertal growth spurt, and other physical and behavioral masculinizing effects (Shirtcliff et al., 2007). In addition, DHEA is also linked to the HPG axis as a precursor to numerous sex hormones and is the originating source of testosterone for females (Belaisch, 2002). Although DHEA is considered both a sex and stress hormone due to its inherent links with both the HPA and HPG axes (Krobath et al., 1999), examining its coupling with other neuroendocrine biomarkers across adolescence will aid in better understanding which axis drives DHEA levels in adolescence.

Neuroendocrine Coupling

In human studies, HPA- and HPG-axis biomarkers are typically examined in isolation, despite strong evidence from basic science and animal models suggesting the presence of functional cross-talk between the two axes (Mastorakos, Pavlatou, & Mizamtsidi, 2006; Rivier, Rivier, & Vale, 1986; Viau, 2002; Viau, Chu, Soriano, & Dallman, 1999; Viau & Meaney, 1991). The animal literature confirms that stress exposure can suppress gonadal functioning in both males and females and that sex hormones can help regulate stress and provide a buffer against the deleterious effects of cortisol (Bingaman et al., 1994; Handa et al., 1994; Johnson, Kamilaris, Chrousos, & Gold, 1992; Lürzel, Kaiser, Krüger, & Sachser, 2011; Tilbrook, Turner, & Clarke, 2000; Toufexis & Wilson, 2012). These findings fit with the basic human literature on healthy adult populations, which typically finds an inverse correlation between cortisol and testosterone (Elias, 1981; Roy, Kirschbaum, & Steptoe, 2003; Zilioli & Watson, 2012). While negative HPA-HPG cross-talk has been most frequently identified in response to situations of stress or challenge, research has yet to explore neuroendocrine coupling over time; however, previous research suggests that negative neuroendocrine coupling may be a developmental phenomenon not fully realized until adulthood.

There are several examples of developmental paradoxes of human adolescence, and the extent to which the HPA and HPG axes work in concert, in opposition, or independently

may be one of them (Buchanan, Eccles, & Becker, 1992; Susman et al., 1997). That is, while theory and empirical evidence supports an inverse association between cortisol and testosterone in adults (Elias, 1981; Viau, 2002), it is not apparent in the literature across adolescence, as positive associations have been identified in both basal (Matchock et al., 2007; Popma et al., 2007; Roy et al., 2003; Scerbo & Kolko, 1994) and reactive states (Budde et al., 2010; Eatough, Shirtcliff, Hanson, & Pollak, 2009). This may be due to the parallel developmental trajectories of the HPA and HPG axes. More specifically, both the HPA and HPG axes are characterized by increased activity across prenatal and postnatal development, quiescence during juvenility or middle childhood, and reactivation during adolescence. If adrenal and gonadal hormones are to rise across adolescence, it would be counterproductive for increases in hormones from one axis to suppress the functioning of the other axis (Shirtcliff & Ruttle, 2010). Moreover, mutually inhibitory sex- and stress-axes do not easily fit with the understanding of adolescence as an inherently stressful developmental stage during which normative increases in activity of both systems may be adaptive (Dahl et al., 1989; Ellis, 2004; Nelson, Leibenluft, McClure, & Pine, 2005). Indeed, research examining cortisol, testosterone, and DHEA in adolescents suggests all three hormones demonstrate stress-reactive properties that would not be possible if increases in one hormone automatically produced decreases in another (Bateup et al., 2002; Eatough et al., 2009; Marceau et al., 2012). If suppression were apparent but minimal enough for puberty to still advance, then the prediction would be that cortisol and/or stress exposure would be associated with later/slower puberty or HPG functioning, but the opposite is typically seen (Ellis, 2004). Although limited animal (Viau, 2002) and human (Matchock et al., 2007) research has found differences in HPA-HPG associations as a function of puberty, important theoretical and methodological limitations have restricted the generalizability of these findings to a human adolescent population.

The Influence of ELS on Neuroendocrine Functioning

Examining normative patterns of HPA-HPG coupling across adolescence is a first step toward understanding how individual differences in such coupling may be calibrated by ELS exposure. A substantial body of research has established that exposure to a non-optimal rearing environment early in life has long-term effects on developing neurophysiological pathways that may have profound implications for later development and health (Gunnar & Quevedo, 2007). While the influence of ELS exposure (e.g., parental depression, marital conflict) on later cortisol levels has been well established (Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002; Essex, Klein, Cho, & Kalin, 2002; Essex, Shirtcliff, et al., 2011; Koss et al., 2011; Koss et al., 2011), the effects of ELS on DHEA and testosterone have been less thoroughly examined. Ellis and Essex (2007) found that parental reports of family functioning and mental health predicted children's accelerated sexual development, including elevated levels of DHEA. Although human research has yet to link exposure to ELS to either concurrent or subsequent altered levels of testosterone, some studies have found that ELS, particularly family upheaval and parental depression, influences pubertal timing, tempo, and development (Belsky et al., 2007; Ellis & Garber, 2000; Tither & Ellis, 2008), likely indirectly illustrating effects of gonadal hormones that advance puberty (Hayward & Sanborn, 2002).

Furthermore, ELS may differentially influence girls' and boys' physiological functioning. Limited research has identified sex differences in the effect of early life stress on children's HPA axis functioning (Hastings et al., 2011); however, similar effects on adolescents' cortisol levels have not been established (Essex, Shirtcliff, et al., 2011), suggesting a need for further research. Regarding the effect of ELS on DHEA or testosterone across adolescence, evolutionary-based models such as psychosocial acceleration theory (Belsky, Steinberg, & Draper, 1991) suggest that familial disruption may influence the development of advanced reproductive strategies, presumably through altered levels of reproductive hormones, and that girls may be particularly susceptible to such psychosocial stress. Extensive research supports this notion, with stressful family relationships, elevated levels of conflict and depression, and father absence all predicting earlier sexual development in girls (Bogaert, 2005; Deardorff et al., 2011; Ellis & Essex, 2007; Ellis & Garber, 2000; Tither & Ellis, 2008). A limited amount of research has examined similar associations in boys, yielding inconclusive results that either suggest comparable (Bogaert, 2005; Ellis & Essex, 2007) or no effects (Belsky et al., 2007) on boys' sexual development; therefore it may be important to consider sex differences in the effect of ELS on neuroendocrine coupling.

Present Study

The present study addresses two major issues. First, we examine the patterns of coupling of cortisol, testosterone, and DHEA from early to middle adolescence. Given that the majority of previous research has focused on cortisol, analyses are conducted with it as the outcome of interest. We hypothesize that, due to the rapidly developing hormonal milieu and novel psychosocial challenges associated with adolescence, young adolescents will display hormone coupling that is tenuous—or even opposite of that demonstrated by adults—but will progress to more adultlike patterns with age. Cortisol-testosterone coupling clearly represents HPA-HPG coupling, and thus we would expect coupling to become progressively more negative, reflecting the tendency for the adrenal and gonadal axes to work in opposition in adults. In contrast, DHEA has links to both the HPA and HPG axes, and different patterns of coupling with cortisol would be expected depending on whether DHEA is linked primarily to the HPA axis (i.e., increasingly positive coupling) or HPG axis (i.e., increasingly negative coupling); therefore we do not hypothesize on the specific direction of cortisol-DHEA coupling.

Second, we investigate whether and how neuroendocrine coupling across adolescence is calibrated by exposure to ELS. This question stems from our prior empirical work showing that ELS influences HPA functioning and development (Essex, Shirtcliff, et al., 2011) and advances pubertal maturation rates (Ellis et al., 2011). If ELS exposure alters HPA-HPG coupling, then this may be a mechanism through which ELS is translated into early sexual maturation. We hypothesize that adolescents exposed to higher levels of stress early in life will exhibit different patterns of neuroendocrine coupling from early to middle adolescence than those not exposed to ELS. Further, given that substantial evidence suggests that psychosocial stress has been shown to result in advanced pubertal development and timing in girls, we anticipate that ELS may elicit altered HPA-HPG coupling patterns only in girls. Given our own (Ellis & Essex, 2007; Essex et al., 2002; Essex, Shirtcliff, et al., 2011) and others' work (Ashman et al., 2002; Ellis & Garber, 2000; Koss et al., 2011; Koss et al.,

2012) that considered the effects of specific types of ELS, we anticipate that the negative effects of ELS on hormone coupling may be driven by high levels of parental depression and family expressed anger.

Method

Participants

Participants are part of the Wisconsin Study of Families and Work (formerly the Wisconsin Maternity Leave and Health Project), a longitudinal study originally designed to assess parental employment leave, family stress, and women's health outcomes during the first postnatal year. A total of 570 women and their husbands/partners were initially contacted during the second trimester of pregnancy through obstetric/gynecology and low income clinics in the Milwaukee (80%) and Madison (20%) Wisconsin geographical areas (for additional details, see Hyde, Klein, Essex, & Clark, 1995). Of the original sample, 560 (98.3%) had eligible live births.

Families living within geographic proximity to the project offices were asked to participate in the saliva collection protocol. Participants were included in analyses if they were able to provide sufficient saliva for the assaying of all three hormones for at least one assessment period; thus, analyses were conducted with 346 individuals. There were no significant differences between the 346 participants and the remainder in terms of parental education, marital or ethnic status, annual family income, or maternal age at recruitment; however, fathers in the participating subsample were slightly older: $M = 31.7$ ($SD = 5.21$) versus 30.7 ($SD = 4.76$), $t(548) = -2.40, p < .05$. Most participating parents identified as Caucasian (89%), were married (96%), and were well-educated (mothers: 1% < high school degree, 42% graduated high school, 38% graduated college, and 18% had postcollege education; fathers were similar). Annual family income was approximately \$47,000 (range = \$7,500 to \$200,000). Parents gave informed consent at each time point; child assent was obtained beginning at age 11. All procedures were reviewed and approved by the University of Wisconsin Institutional Review Board.

Measures

Data on ELS were collected during infancy (child age 1, 4, and 12 months) and preschool (child age 3.5 and 4.5 years). Children's hormone levels were assessed in the summers following grade 5 ($N = 297$; M age = 11.19, range = 10.5 – 12.3), grade 7 ($N = 306$; M age = 13.22, range = 11.6 – 14.2), and grade 9 ($N = 273$; M age = 15.50, range = 14.8 – 16.5).

Pubertal development.—Puberty was measured using a multimethod, multi-informant approach. Mothers (ages 11 and 13) and youths (ages 11, 13 and 15) completed a Tanner-stage questionnaire measure based on description and visual inspection of line drawings (Morris & Udry, 1980), and mothers (ages 11, 13 and 15) completed the Pubertal Development Scale (Petersen, Crockett, Richards, & Boxer, 1988). For a more detailed description, see Ellis et al. (2011).

Age.—Age was included in analyses to determine if patterns in hormone coupling changed across adolescence. Age was coded as Years Since School Entry to center development on a meaningful event and take into consideration important experience-based social transitions (Shirtcliff & Essex, 2008).

Adolescent salivary hormones.—Within each of the three assessments, adolescents were asked to collect saliva for three consecutive days across three target collection times: (1) shortly after waking (before brushing teeth or eating breakfast); (2) at a participant-set target between 3:00 PM and 7:00 PM (prior to dinner); and (3) at a participant-set target time prior to bedtime. Given that cortisol, testosterone, and DHEA all demonstrate circadian profiles characterized by high morning levels (Guyton & Hall, 1996) and samples collected shortly after waking are less likely to be influenced by a variety of factors (e.g., social interactions, exercise) than samples taken later in the day, morning samples were assayed for all three hormones. Participants were asked to freeze samples immediately after collection; once all samples for a particular assessment were complete, research staff collected the samples and transported them to the laboratory where they were stored at -80°C until assaying. If all three collection tubes contained a sufficient volume of saliva, the two morning tubes collected closest in time were assayed. Hormones were assessed in duplicate using well-established, salivary enzymeimmunoassay kits (Salimetrics, State College, PA). Raw cortisol, testosterone, and DHEA scores were log-transformed and extreme values were winsorized to normalize distributions. Previous analyses revealed that neither BMI nor medication usage systematically impacted salivary cortisol (Shirtcliff et al., 2012); however, given the likely effect of oral steroids on cortisol levels (Masharani et al., 2005), data for one child taking prednisone at one assessment were omitted. Analyses were conducted to examine the impact of BMI and medication usage on DHEA and testosterone in the present study, and no systematic associations were detected.

Hormone assays.—For cortisol, mean intra-assay and interassay coefficients of variation (CVs) were 3.8% and 7.4%, respectively, with a lower limit of sensitivity of $0.02\text{ }\mu\text{g/dL}$. For DHEA, mean intra-assay and interassay CVs were 4.9% and 3.45%, respectively, with a lower limit of sensitivity of 10 pg/mL . For testosterone, mean intra-assay and interassay CVs were 5.0% and 7.35%, respectively, with a lower limit of sensitivity of 1.5 pg/mL .

Early life stress.—As in our prior work (e.g., Ellis, Essex, & Boyce, 2005; Essex, Klein, Slattery, Goldsmith, & Kalin, 2010), separate maternal- and paternal-report stress scores were constructed from five domains: (a) depression symptoms (Radloff, 1977); (b) family expressed anger, including marital conflict (Barnett & Marshall, 1989) and general anger expression (Halberstadt, 1986; Spielberger, Krasner, Solomon, & Janisse, 1988); (c) parenting stress (Abidin, 1986; Block, 1965); (d) role overload (Abidin, 1986; Barnett & Marshall, 1989); and (e) financial stress (Essex et al., 2002). For each parent separately, reports of the five stress domains were combined using Principal Components Analysis (PCA) in infancy (ages 1, 4 and 12 months) and preschool (ages $3\frac{1}{2}$ and $4\frac{1}{2}$ years). The first component of each PCA accounted for more than 50% of the variance; all loadings exceeded .50. For each parent, factor scores were correlated across time ($r_s > .65$, $p_s < 0.001$) and thus averaged within parent before averaging mother and father scores together. To

probe ELS findings in secondary analyses, separate measures for the five stress domains were also computed similarly, i.e., for each domain, mean scores from infancy and preschool were averaged for each parent, then mother and father scores were averaged.

Major Analytic Strategy

Hierarchical linear modeling (HLM) was selected as the major analytic strategy because of its ability to measure the covariation of variables over time and model complex growth curves based on multiple observations, nested within an individual. The research questions were addressed using a 3-level HLM model (for more information, see Bryk & Raudenbush, 1992) that separated within-individual ($n = 1706$ samples), wave-to-wave ($n = 862$ waves), and between-individual ($N = 346$ participants) sources of cortisol variability in order to explore linear and non-linear patterns of neuroendocrine coupling as well as predictors of coupling.

HLM Model Associated with Hormone Covariation across Adolescence and the Influence of ELS

Level 1: Within-individual covariation of cortisol, testosterone, and DHEA.—

Morning cortisol level was entered as the outcome variable ($N = 1706$; maximum of 2 samples/ wave over 3 waves of collection). Corresponding morning levels of testosterone and DHEA were also entered at Level 1 thereby capturing whether HPA and HPG functioning covaried concurrently. In this manner, testosterone level or DHEA level is a predictor of cortisol level at that sample collection within an individual. A hierarchical model separates within- from between-individual or -wave sources of variability (Hruschka, Kohrt, & Worthman, 2005), so that this covariation is not merely a significant correlation between the hormones, but rather provides the opportunity to examine coupled intra-individual processes beyond correlated change scores (Sliwinski, Smyth, Hofer, & Stawski, 2006).

While instructions specified saliva collection immediately upon awakening, compliance varied. HLM is able to model the influence of time in the analyses instead of excluding participants who provided samples later than instructed. As such, time variables (i.e., time since waking and time since waking squared) were entered in order to control for the influence of the diurnal rhythm on hormone level.

Level 2: Assessment-to-assessment hormonal covariation.—The growth-curve model simultaneously includes a Level 2 hierarchy that captures cortisol measures within each wave of data collection ($N = 862$; maximum of 3 waves per person). Cortisol-DHEA and cortisoltestosterone covariation (distinguished in the Level 1 model) are outcomes of interest at each wave, with the sign of the score indicating the direction of the coupling and the absolute value indicating the strength of that association. To examine the developmental trajectory of HPA- HPG-axis maturation across waves, age (i.e., Years Since School Entry) is entered as a fixed predictor of hormonal covariation. Individual and combined measures of puberty were also examined to determine if hormone covariation was better predicted by progression through pubertal stages. Fixed quadratic terms of age and pubertal development were included separately to test if hormone coupling across the four-year period of early to

middle adolescence was nonlinear (Schreiber et al., 2006). Results from Level 1 and Level 2 analyses address the first research question regarding patterns of hormone covariation across adolescence.

Level 3: Between-individual predictors of hormone covariation.—Level 3 captures between-individual variability or stability in morning cortisol levels and hormone coupling across time ($N = 346$). Child sex, ELS, and the two-way sex $\times$ ELS interaction are included as fixed predictors of the outcomes isolated from the Level 1 and Level 2 components of the HLM: cortisol level, cortisol covariation with DHEA and testosterone, the trajectory of morning cortisol levels across adolescence, and the pattern of hormone covariation across adolescence. Results from the Level 3 analyses address the second research question, i.e., whether ELS influences patterns of hormone covariation across early to middle adolescence. See Table 1.

Next, using the same model outlined above, secondary analyses using the five separate components of ELS (i.e., parental depression, family anger, parenting stress, role overload, financial stress) were separately examined to determine which components were influencing the ELS findings. Given the modest to strong associations between the individual components, significant predictors were considered to present joint, rather than independent, effects.

Descriptive Statistics

Preliminary analyses reveal there was significant variability within children's cortisol, $\chi^2(516) = 859.35, p < .001$, indicating that there were individual differences in morning cortisol levels. Two time-related variables (time since waking: TSW; the quadratic function of time since waking: TSW²) were included in the model and both were found to be significant (TSW: $B = .062, t = 2.25, p < .05$; TSW²: $B = -.046, t = -4.58, p < .001$). According to this base model, within-wave (i.e., day-to-day) fluctuations in morning cortisol accounted for 26.9% of the total variance in cortisol, wave-to-wave cortisol fluctuations accounted for 17.4% of the total variance, and between-individual fluctuations accounted for 55.7% of the total variance in cortisol levels.

Results

Question 1: Neuroendocrine Coupling

Morning levels of DHEA and testosterone were entered as Level 1 predictors of morning levels of cortisol. Overall, DHEA was positively associated with cortisol measured concurrently within each individual, ($B = .282, t = 10.30, p < .001$), such that when individuals displayed higher levels of cortisol, they also displayed higher levels of DHEA. Conversely, testosterone was not a significant predictor of average concurrent levels of cortisol ($B = .053, t = 1.54, p = \text{ns}$), suggesting lack of a consistent overall pattern of cortisol-testosterone coupling.

To examine whether coupling changed across development, age and age-squared were included as Level 2 predictors to model changes in the trajectory of cortisol and hormone coupling across adolescence. Results indicated that while an initial overall positive

association was found for cortisol-DHEA coupling, the inclusion of the age variables as predictors of change over time showed that there was weaker initial coupling ($B = -1.655$, $t = -1.94$, $p = .05$) that became more positive over time (age: $B = .429$, $t = 1.91$, $p = .06$; age²: $B = -.021$, $t = -1.48$, $p = \text{n.s.}$). See Figure 1.

Age effects were also detected for cortisol-testosterone coupling changes (intercept: $B = 3.247$, $t = 3.22$, $p < .01$; age: $B = -.772$, $t = -2.97$, $p < .01$; age²: $B = .045$, $t = 2.75$, $p < .01$). See Figure 2. Initially there was a positive coupling of cortisol and testosterone within individuals; however, at age 13, cortisol and testosterone were *negatively*, albeit weakly, coupled. At age 15, cortisol and testosterone were not coupled at this level of the model (but see Level 3 results below). Measures of pubertal development were not significant predictors of changes in cortisol-testosterone or cortisol-DHEA coupling across adolescence ($p > .05$).

Question 2: Influence of ELS on Neuroendocrine Coupling

At Level 3, child sex, ELS, and their interaction were entered into the model to examine whether adolescents exposed to ELS displayed altered hormone coupling patterns. There was a main effect of ELS on cortisol-DHEA coupling ($B = -2.59$, $t = -2.61$, $p = .01$) that varied as a function of age (age: $B = .700$, $t = 2.67$, $p < .01$; age²: $B = -.046$, $t = -2.73$, $p < .01$), suggesting that the effect of ELS on cortisol-DHEA coupling differed over time. When they were young, non-exposed individuals demonstrated tenuous, positive cortisol-DHEA coupling that became progressively stronger with age. Conversely, rather than demonstrating a linear progression of cortisol-DHEA coupling, individuals exposed to ELS demonstrated weak positive coupling at age 11 that became much stronger by age 13 and, unlike the non-exposed adolescents, this coupling did not then become stronger at age 15. See Figure 4.

When considering cortisol-testosterone coupling patterns, a significant sex $\times$ ELS effect appeared ($B = 6.207$, $t = 2.22$, $p < .05$) that varied as a function of age (age: $B = -1.519$, $t = -2.10$, $p < .05$; age : $B = .089$, $t = 2.00$, $p < .05$). Further examination revealed that this effect was unique to girls and no effect of ELS on cortisol-testosterone coupling was present for boys. More specifically, at age 11 non-exposed girls demonstrated positive coupling of cortisol with testosterone, which progressively switched to become an inverse association by age 15. Conversely, girls exposed to ELS demonstrated tighter positive coupling initially, which then switched to negative coupling by age 13; unlike the non-exposed adolescents, this coupling did not then become stronger at age 15. See Figure 4.

In secondary analyses, the same model was employed to separately examine each component of ELS to examine which aspects of the stress composite might be driving the observed findings. These analyses revealed that parental depression and family anger were the primary drivers of these associations, and that negative parenting, role overload and financial stress demonstrated no predictive ability on their own.

Discussion

The present study investigated patterns of neuroendocrine coupling across adolescence and the moderating role of exposure to ELS. Overall, results supported our hypothesis that adolescents would demonstrate changes in the patterns of neuroendocrine coupling as they

matured through adolescence. Cortisol and DHEA exhibited weak positive coupling at age 11 that developed into more adultlike strong positive coupling over time. Conversely, cortisol and testosterone demonstrated an initially positive coupling pattern that was more negatively coupled at ages 13 and 15. Results also supported our hypotheses that exposure to ELS would impact neuroendocrine coupling and that the effect of ELS may differentially influence girls' versus boys' coupling. More specifically, exposure to ELS (especially parental depression and family anger) resulted in more adultlike, positive cortisol-DHEA coupling at age 13 that did not become more tightly positively coupled at age 15. Among girls only, ELS exposure also resulted in more negative cortisol-testosterone coupling at age 13 that did not become more tightly negatively coupled at age 15.

Our investigation supports previous research suggesting that a distinct pattern of neuroendocrine coupling may be present in adolescence. While adults generally display a positive association between cortisol and DHEA (Hucklebridge, Hussain, Evans, & Clow, 2005) and an inverse association between cortisol and testosterone (Bingaman et al., 1994; Elias, 1981; Handa et al., 1994), findings from the present study suggest these patterns are not present in early adolescence but develop gradually over the next few years and possibly beyond. Preliminary evidence of unique hormone activity in adolescence was identified in rats (Viau, 2002), but the applicability of this model to human development was unclear due to different maturation processes in humans (e.g., adrenarche). More recently, Matchock and colleagues (2007) explored interactions between cortisol, testosterone and DHEA as a function of pubertal status and identified some evidence of unique patterns in adolescence, but small sample size and cross-sectional, inter-individual measurement of hormones may have limited their ability to detect stronger effects. By taking a longitudinal, intra-individual approach to studying neuroendocrine coupling, the present study provides support of the existence of unique hormone activity from early to middle adolescence and extends prior research by framing neuroendocrine coupling in a developmental context, demonstrating progression to patterns that become more adultlike.

Cortisol-DHEA.

Overall, results suggest a positive coupling of cortisol and DHEA that is more tenuous at age 11 but increases over time. This association may be influenced by two different processes. First, this pattern of increased positive coupling over time may be reflective solely of HPA-axis activity, with weak earlier coupling reflecting the recent activation of the HPA axis and more positive coupling over time reflecting subsequent maturation. An alternative explanation is that this shift may be indicative of a developmental switch in the axis driving DHEA levels. Consistent with the notion that DHEA has dual roles as both a stress hormone and as a sex hormone (Parker, 1999; Shirtcliff et al., 2007; Tung, Lee, Tsai, & Hsiao, 2004), weaker early coupling may reflect the fact that DHEA is still operating as a sex hormone following its integral role in prompting the commencement of adrenarche. However, adrenal androgens such as DHEA are relatively weak compared to gonadal-based androgens, such as testosterone. Therefore, as adolescents grow older and the HPG axis becomes more developed, gonadal androgens supersede the androgenic properties of DHEA to guide physical development (Dorn et al., 2006), possibly prompting DHEA to begin functioning less like a sex hormone and more like a stress hormone. Positive cortisol-DHEA coupling

later in adolescence mirrors findings in adults and may reflect the shared adrenal origin of both hormones (Grumbach, 2002; Oskis, Clow, Thorn, Loveday, & Hucklebridge, 2012; Shirtcliff, Dahl, & Pollak, 2009; Wolf & Kirschbaum, 1999) as well as the protective effect of DHEA against the catabolic effects of cortisol (Goodyer, Park, Netherton, & Herbert, 2001).

Cortisol-Testosterone.

Positive cortisol-testosterone coupling patterns were detected in early adolescence. Although positive coupling is not consistent with studies in adults (Elias, 1981; Hoogeveen & Zonderland, 1996; MacAdams, White, & Chipps, 1986; Morgan et al., 2000), it is consistent with findings from a previous investigation showing positive associations of cortisol with sex hormones in adolescence, particularly early adolescence (Matchock et al., 2007). Interpreted within a developmental framework, positive early basal cortisol-testosterone coupling may be a part of normative pubertal development. If the HPA and HPG axes were consistently mutually inhibitory, the increased HPA-axis activity due to adrenarche would suppress the activity of the HPG axis and pubertal development (e.g., Ellis et al., 2011). Positive cortisol-testosterone coupling has also been observed in situations of potential mate-acquisition, suggesting flexibility in axis coupling during circumstances in which it is advantageous for the individual to be sexually mature even against a background of stress and/or cortisol release (Gettler, McDade, & Kuzawa, 2011; Lopez, Hay, & Conklin, 2009; Roney, Lukaszewski, & Simmons, 2007). While positive cortisol-testosterone coupling may be advantageous in early adolescence and certain social situations, a transition to more negative coupling would be anticipated as negative coupling is generally considered to be adaptive in adulthood (i.e., suppression of the HPG axis in times of stress leads to the conservation of energy and increased chances of survival; (Rabin, Gold, Margioris, & Chrousos, 1988). We speculate that maturation of the gonads (i.e., gonadarche) may be driving these developmental patterns. These results tentatively suggest that initially both the HPA and HPG axes are developing and maturing, resulting in positive coupling but, as the axes mature over time, their cross-talk develops into a more mutually inhibitory pattern. While the degree of negative coupling an individual demonstrates outside of a stressful situation may vary according to basal levels of cortisol and testosterone, progression away from a positive association would be anticipated (Viau, 2002). However, certain studies suggest that a negative association between cortisol and testosterone may not necessarily be present in adults (Mehta, Jones, & Josephs, 2008; Mehta & Josephs, 2010), suggesting additional research is necessary in this domain. Importantly, pubertal stage was not a significant moderator of neuroendocrine coupling above and beyond age. This is consistent with other work suggesting that cortisol trajectories are associated with age-related development more so than puberty per se (Essex, Shirtcliff, et al., 2011; Shirtcliff et al., 2012; Shirtcliff & Essex, 2008) and that adrenarche, while considered a pubertal event, is independent of pubertal timing or development (Dorn et al., 2006; Havelock, Auchus, & Rainey, 2004).

The Influence Effect of ELS on Neuroendocrine Coupling.

In addition to identifying normative longitudinal patterns of neuroendocrine coupling from early to middle adolescence, the findings demonstrate the effects of exposure to ELS on

these coupling patterns. Adolescents exposed to low levels of ELS demonstrated weak positive cortisol-DHEA coupling that gradually progressed to strong positive coupling by midadolescence. This contrasts with the progression of cortisol-DHEA coupling in adolescents exposed to ELS, which after demonstrating initial weak coupling became more tightly positively coupled by age 13 but then (unlike their non-exposed counterparts) did not demonstrate a further increase at age 15. In other research, more pronounced positive coupling of multiple HPA biomarkers has been found in prepubertal children exposed to child abuse (De Bellis et al., 1999); however, work in older adolescent females suggests that imbalanced cortisol/DHEA ratios may be indicative of risk (Pajer et al., 2006). Thus, both patterns may be atypical, albeit on different time courses. While a positive association between cortisol and DHEA is generally considered to be a normative coupling pattern in older, more pubertally advanced adolescents (Netherton, Goodyer, Tamplin, & Herbert, 2004) and adults (Hucklebridge et al., 2005), this pattern may not necessarily be appropriate for younger individuals (Netherton et al., 2004). Later in adolescence and beyond, when a positive association is normative, an imbalance of the two hormones may be representative of the inability of DHEA to mount a counterresponse to the negative effects of cortisol, possibly indicative of adrenal dysfunction and increased susceptibility to certain mental health problems, such as depression (e.g., Goodyer et al., 1996; Goodyer et al., 2003).

Cortisol-testosterone coupling was also influenced by ELS exposure; however, this effect was only present in girls. While non-exposed girls demonstrated gradual progression of weak positive to weak negative cortisol-testosterone coupling by age 15, girls exposed to ELS displayed stronger positive cortisol-testosterone coupling in early adolescence that switched to negative coupling earlier (i.e., by age 13) that did not become more pronounced at age 15. Although research has yet to empirically understand the implications of altered HPA-HPG interactions as a function of ELS, the Life History theory suggests that this pathway may result in early sexual maturation. As described by Ellis (2004), although energetics or stress suppression models suggest an inhibitory role of stress exposure on pubertal and adolescent development, development is typically *accelerated* in adolescents exposed to such early life stressors. The Life History literature does not identify an underlying biological mechanism to explain accelerated development, but Ellis (2004) describes several plausible mechanisms, ending with a speculation that co-activation of stress hormones and sex hormones may link ELS exposure to adolescent maturation rates. That is, exposure to negative life influences may calibrate the HPA axis early in development; then, at the beginning of adolescence when the systems are reactivated, altered HPA activity may influence HPG functioning, resulting in accelerated maturation. The present study identifies the presence of more adult-like patterns of cortisol-testosterone coupling earlier in development in girls exposed to ELS, which can be indicative of advanced sexual development, possibly providing empirical support for the Life History theory. Moreover, negative cortisol-testosterone coupling in early adolescence was found in high risk boys who displayed high levels of aggressive behavior (Popma et al., 2007), possibly suggesting that early inverse HPA-HPG coupling may be linked to behavior and/or mental health problems. Although speculative, negative HPA-HPG coupling at an early age may be a sign of early maturation which is considered to be generally disadvantageous (Bjorklund, 1997).

Conversely, boys' cortisol-testosterone coupling was not significantly impacted by ELS, and both ELS exposed and non-exposed boys demonstrated mostly tenuous or positive cortisoltestosterone coupling across adolescence. Positive coupling has also been identified in studies of adults (Gettler et al., 2011; Mehta et al., 2008) and undergraduates (Liening, Stanton, Saini, & Schultheiss, 2010; López et al., 2009; Mehta et al., 2008; Mehta & Josephs, 2010; Roney et al., 2007; Zilioli & Watson, 2012), often in the context of challenge or sexually motivated circumstances, and sometimes this effect was found in males but not females (Liening et al., 2010). These studies may suggest that individuals may be more prone to HPA-HPG co-activation in certain situations and/or that positive coupling may present for an extended period of time, particularly in males. It should also be noted that the majority of studies finding an inverse association between cortisol and testosterone in adults involve stressful situations, underscoring the need for additional work examining HPA-HPG associations under basal conditions.

Secondary analyses revealed that exposure to parental depression and family anger were the components of ELS exerting significant effects on neuroendocrine coupling. Angry, destructive parental conflict undermines children's sense of security and can leave children feeling vulnerable and unsure about the future of their family (Cummings, Schermerhorn, Davies, Goeke-Morey, & Cummings, 2006), and parental depression has been shown to negatively influence children's developmental and emotional needs (Arsenio, Sesin, & Siegel, 2004). Both of these experiences can create cues within the early environment of instability or unpredictability, two key dimensions thought to powerfully adjust children's neuroendocrine developmental trajectories (Cummings & Davies, 1994; Cummings et al., 2006).

Our findings extend previous empirical research examining the effect of ELS on adolescent neuroendocrine functioning and provide a potential mechanism through which ELS may theoretically result in advanced sexual maturation. Our previous work suggests that exposure to ELS leads to altered patterns of HPA-axis function in adolescence (Essex, Shirtcliff, et al., 2011) as well as early sexual development, including early adrenarche and corresponding levels of DHEA (Ellis & Essex, 2007). However, rather than examining a single biomarker, here we took a broader approach. Dual-system neuroendocrine coupling across adolescence identifies within-individual, multisystem processes and highlights the joint contributions of interrelated systems while simultaneously considering adolescence as a dynamic life stage. The organizational effects of early influences on neuroendocrine *coupling* rather than on the activity of a single system may have profound implications for the field of neuroendocrinology as well as understanding pathways to developmental psychopathology. Furthermore, the results of the present study highlight a possible underlying biological mechanism—calibration of the interconnected adrenal and gonadal systems—responsible for shaping life-history strategies and psychosocial acceleration.

This study is an important step towards a more holistic and integrative approach to studying the effect of early life influences on dynamic neuroendocrine systems, but is not without its limitations. The sample has a preponderance of white, middle-class, intact families. While this was an excellent sample for determining the normative coupling of neuroendocrine biomarkers across adolescence, it is restricted to a range of ELS typical of a community

sample. Examining similar associations in samples with extreme forms of early life influences may be informative. Second, although the present study examined hormones associated with the HPA and HPG axes, examining the coupling of other highly interactive systems may be important for understanding adolescent development; for example, HPA and immune systems are intricately connected (Daruna, 2004; Elenkov & Chrousos, 1999) and both systems can be organized by ELS exposure (Shirtcliff, 2009; Shirtcliff, Coe, & Pollak, 2009; Shirtcliff & Ruttle, 2010; Wismer Fries, Shirtcliff, & Pollak, 2008). Third, the majority of studies of HPA-HPG associations have examined coupling under situations of stress. Given the paucity of literature examining neuroendocrine coupling of basal hormones, additional research should be conducted in this area to determine the interplay of hormones under non-stressful circumstances. Finally, atypical neuroendocrine functioning has been repeatedly linked to altered mental health (reviewed in Gunnar & Vasquez, 2006; Trickett, Negri, Ji, & Peckins, 2011). Future research should determine the additional value that examining multiple systems provides in terms of clarifying the divergent hormone-behavior associations plaguing the literature.

Examining dual-system coupling, rather than a single physiological system, may provide a more holistic view of development and health, particularly in a transitional life stage such as adolescence (Bauer, Quas, & Boyce, 2002; Hastings et al., 2011). Identifying typical patterns of HPA-HPG coupling across adolescence is an essential first step to establishing developmentally *atypical* coupling patterns that may denote vulnerability or risk for the development of mental or physical health problems. Furthermore, the effect of ELS on hormone coupling suggests a possible mechanism through which negative ELS may impact pubertal development and confer risk for future negative outcomes.

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Figure 1.
Cortisol-DHEA coupling across ages 11, 13, and 15.

Figure 2.
Cortisol-testosterone coupling across ages 11, 13, and 15.

Figure 3.
The moderating effect of exposure to early life stress (ELS) on cortisol-DHEA coupling.

Figure 4.
The effect of exposure to early life stress (ELS) on cortisol-testosterone coupling in girls (a) and boys (b).

Table 1

Illustration of the Three Levels of the HLM

Level 1:	$\text{Cort} = \pi_0 + \pi_{1\text{DHEA}} + \pi_{2\text{testosterone}} + \pi_{3\text{TSW}} - \pi_{4\text{TSWsq}} + e$
Level 2: [developmental change]	$\pi_0 = \beta_{00} + \beta_{01\text{age}} - \beta_{02\text{agesq}} + R_0$ $\pi_{1\text{DHEA}} = -\beta_{10} + \beta_{11\text{age}} - \beta_{12\text{agesq}}$ $\pi_{2\text{Testo}} = \beta_{20} - \beta_{21\text{age}} + \beta_{22\text{agesq}}$ $\pi_{3\text{TSW}} = \beta_{30}$ $\pi_{4\text{TSWsq}} = -\beta_{40}$
Level 3 :	
[set-point in cortisol level]	$\beta_{00} = \gamma_{000} + \gamma_{001\text{sex}} - \gamma_{002\text{ELS}} + \gamma_{003\text{sex*ELS}} + U_{00}$
[cross-level interactions age on cortisol level]	$\beta_{01\text{age}} = \gamma_{010} - \gamma_{011\text{sex}} + \gamma_{012\text{ELS}} + \gamma_{013\text{sex*ELS}}$ $\beta_{02\text{agesq}} = -\gamma_{020} + \gamma_{021\text{sex}} - \gamma_{022\text{ELS}} + \gamma_{023\text{sex*ELS}}$
[set-point in cortisol-DHEA coupling]	$\beta_{10} = -\gamma_{100} + \gamma_{101\text{sex}} - \gamma_{102\text{ELS}} - \gamma_{103\text{sex*ELS}} + U_{10}$
[cross-level interaction with age on cortisol-DHEA coupling]	$\beta_{11\text{age}} = \gamma_{110} - \gamma_{111\text{sex}} + \gamma_{112\text{ELS}} + \gamma_{113\text{sex*ELS}}$ $\beta_{12\text{agesq}} = -\gamma_{120} + \gamma_{121\text{sex}} - \gamma_{122\text{ELS}} - \gamma_{123\text{sex*ELS}}$
[set-point in cortisol-testosterone coupling]	$\beta_{20} = \gamma_{200} + \gamma_{201\text{sex}} + \gamma_{202\text{ELS}} + \gamma_{203\text{sex*ELS}} + U_{20}$
[cross-level interaction with age on cortisol-testosterone coupling]	$\beta_{21\text{age}} = -\gamma_{210} - \gamma_{211\text{sex}} - \gamma_{212\text{ELS}} - \gamma_{213\text{sex*ELS}}$ $\beta_{22\text{agesq}} = \gamma_{220} - \gamma_{221\text{sex}} + \gamma_{222\text{ELS}} + \gamma_{223\text{sex*ELS}}$
[fixed effect of TSW]	$\beta_{30\text{TSW}} = \gamma_{300}$
[fixed effect of TSWsq]	$\beta_{40\text{TSWsq}} = \gamma_{400}$

Note. Significant coefficients in the final model are denoted in **bold**. Trend coefficients in the final model are denoted in *italics*.