Biological Stress Regulation in Female Adolescents: A Key Role for Confiding

Abstract

Attachment behaviors play a critical role in regulating emotion within the context of close relationships, and attachment theory is currently used to inform evidence-based practice in the areas of adolescent health and social care. This study investigated the association between female adolescents' interview-based attachment behaviors and two markers of hypothalamic-pituitary-adrenal (HPA) axis activity: cortisol and dehydroepiandrosterone (DHEA). Unlike the classic stress hormone cortisol, there is very limited investigation of DHEA— a quintessential developmental hormone—in relation to attachment, especially in adolescents. Fifty-five healthy females mean age 14.36 (± 2.41) years participated in the Attachment Style Interview. A smaller cortisol awakening response was related to anxious attachment attitudes, including more fear of rejection, whereas greater morning basal DHEA secretion was only predicted by lower levels of reported confiding in one's mother. These attachment-hormone relationships may be developmental markers in females, as they were independent of menarche status. These findings highlight that the normative shifts occurring in attachment to caregivers around adolescence are reflected in adolescents' biological stress regulation. We discuss how studying these shifts can be informed by evolutionary-developmental theory.

Keywords

Adolescence, Attachment, CAR, Cortisol Awakening Response, DHEA, HPA Axis

Introduction

Attachment relationships are believed by many researchers and practitioners to be vitally important in young people's healthy development (Guttmann-Steinmetz & Crowell, 2006; Sroufe, 2005). This is especially relevant in the United Kingdom, which faces the dilemma of being placed at the bottom of a list of 21 developed countries in terms of child and adolescent well-being (UNICEF, 2007). The UK, like the United States, has a social policy agenda that places the relationship between children and their caregivers at the heart of its concern. Attachment theory, as originally outlined by Bowlby (1969), has significant implications for policy and practice, where it is currently being used to inform evidence-based practice concerning young people, particularly in the areas of adoption, fostering and social care (Department for Education and Skills, 2006; Maxwell & Cook, 2014). The theory not only provides a powerful framework for understanding relationships (for example, support access and parenting capacity), but it sheds light regarding the linkages with early parenting experiences and the subsequent risks for a range of mental and physical health outcomes (for example see, Bifulco, Moran, Jacobs, & Bunn, 2009; Fagundes, Bennett, Derry, & Kiecolt-Glaser, 2011; Puig, Englund, Simpson, & Collins, 2013). Recent evidence suggests that early attachment history may contribute to increased internalizing symptoms for girls during the transition to adolescence (see Milan, Zona, & Snow, 2013).

Adolescence represents a watershed period in terms of attachment relationships. The bonds that young children are biologically disposed to form with proximate caregivers to protect them in times of stress, threat and danger undergo dramatic shifts during this period of development (Brown & Wright, 2001). The typical adolescent quest for greater autonomy, together with the challenge or rejection

of parental support is part of an active and purposeful reorganization of the hierarchy of attachment figures (Allen & Land, 1999; Moretti & Peled, 2004; Noom, Deković, & Meeus, 1999; Rosenthal & Kobak, 2010). Developing autonomy from parents, or individuating, is a fundamental psychosocial task of adolescence identified by developmental theorists (for example Erikson, 1968). From adolescence forward, one dimension of attachment behavior suggested to be especially important is the ability to disclose and confide in a clear direct way about attachment needs and fears (Johnson, Makinen, & Millikin, 2001). Confiding in a close other is the analogue of the hallmark attachment behavior of seeking proximity to a caregiver in childhood and using caregivers as a safe haven to regulate feelings of insecurity and distress (Bifulco, Moran, Ball, & Bernazzani, 2002; Freeman & Brown, 2001; Johnson et al., 2001). The importance of confiding as an attachment behavior is echoed in recent policy initiatives, with the UK Department for Education and Skills (2006) highlighting the need for young people to "… build secure attachments – this evidenced through confiding relationships and should be assessed through exploring the existence of supportive relationships and capacity to share difficulties and accept help" (p. 48).

Attachment behaviors can be understood across multiple levels of analysis—from the psychological to the biological (Clulow, 2007; Diamond, 2001). Compared to childhood and adulthood, much less is known about the interplay between social relationships and biological regulation during adolescence (Hostinar, Sullivan, & Gunnar, 2014; Murray-Close, 2013; Scott, Briskman, Woolgar, Humayun, & O'Connor, 2011). Bowlby's (1969) original concept of attachment theory described a bio-behavioral system that is activated in times of stress, i.e. when there is a real or perceived threat to a person's sense of felt security (Sbarra & Hazan, 2008). Our

body's primary stress-mediating system, the hypothalamic-pituitary-adrenal (HPA) axis is most widely known for secreting the hormone cortisol. Cortisol's functions as a glucocorticoid include the regulation of energy metabolism, vascular activity, and inflammatory and immune responses (Heaney, Phillips, & Carroll, 2012). There is a wealth of evidence to support cortisol's role as a quintessential stress hormone, and it is perhaps especially pertinent to attachment that this hormone is activated during socio-evaluative and interpersonal threat (Dickerson & Kemeny, 2004). Dysregulated basal levels of cortisol, especially the burst of cortisol secretion that occurs around 30-45 min following awakening (cortisol awakening response: CAR), have been found in females categorized as anxious-insecurely attached (Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2011; Quirin, Pruessner, & Kuhl, 2008). There is also evidence linking attachment anxiety to reduced cell concentration in the left hippocampus, which may underpin aberrant HPA axis function in those with anxious attachment (Quirin, Gillath, Pruessner, & Eggert, 2010). Relatedly, rejection sensitivity and the associated fear of negative social evaluation have been shown to predict a flatter CAR in women (Tops, Riese, Oldehinkel, Rijsdijk, & Ormel, 2008).

These findings suggesting that attachment anxiety may have costs for biological stress regulation are buttressed by recent work investigating basal levels of cortisol in conjunction with reactivity to attachment-specific stressors. For example, Hicks and Diamond (2011) found that only females high in attachment anxiety had a blunted CAR following a relationship dispute the previous evening and Jaremka et al. (2013) found that anxious attachment predicted higher cortisol levels across the day following discussion of marital problems. There is limited research on basal cortisol secretion and attachment earlier in the lifespan, especially in females. Rifkin-Graboi (2008) examined both CAR and cortisol response across the day in young adult males,

but only found an association between attachment anxiety and cortisol during the afternoon. No equivalent research has been carried out with adolescents.

Cortisol is not the only hormone secreted by the HPA axis and relevant to the human stress response. The physiological patterns of the biological stress system regulate variation in a wide range of adaptive processes and behaviours including growth and metabolism, reproductive status and fertility, and a key HPA axis hormone for this is dehydroepiandrosterone (DHEA). DHEA is considered a precursor for several other hormones with androgenic activity (Kroboth, Salek, Pittenger, Fabian, & Frye, 1999) and girls, unlike boys, reach their lifetime peak of DHEA during adolescence (Angold, 2003; Netherton, Goodyer, Tamplin, & Herbert, 2004; Shirtcliff, Zahn-Waxler, Klimes-Dougan, & Slattery, 2007). A physiological role for DHEA other than as a precursor to sex hormones remains to be well-defined (Buvat, 2003; M. D. Johnson, Bebb, & Sirrs, 2002). Like cortisol, though, DHEA also plays a role in modulating immune function (Kroboth et al., 1999). The rise and decline of DHEA with episodes of development suggests a link between DHEA and the process of physical maturation and, potentially, ageing more generally, particularly for females (Labrie, Belanger, Cusan, & Candas, 1997). Findings based on research utilising DHEA supplements in aging populations to improve psychological parameters, such as well-being, cognition and memory contribute to this idea, although results between studies are inconsistent (for example, Barnhart et al., 1999; Flynn, Weaver-Osterholtz, Sharpe-Timms, Allen, & Krause, 1999; Morales, Nolan, Nelson, & Yen, 1994; Wolf, Kudielka, Hellhammer, Hellhammer, & Kirschbaum, 1998; Wolf et al., 1997). Some research has found blunted levels of DHEA in the morning to be associated with adolescent depression (Goodyer et al., 1996).

More often than not, though, in adolescent research DHEA has largely either been studied in the context of the cortisol-DHEA ratio (Goodyer, Herbert, & Tamplin, 2003) or in reactivity paradigms (for example, Shirtcliff et al., 2007; Wemm, Koone, Blough, Mewaldt, & Bardi, 2010). Interestingly, the cross-sectional developmental associations found involving DHEA appear specific to stressors/challenges involving a social component (Marceau et al., 2014). For example, DHEA was found to be responsive to a parent-adolescent conflict interaction task (Shirtcliff et al., 2007). However, there has been limited investigation of DHEA operating as a puberty-related hormone rather than as a stress hormone, in relation to close, social relationships in adolescents.

Given its role in both development and social interactions, DHEA might therefore be an ideal candidate to shed light on the broader function of the biological stress system; some consider the biological stress system to have dual functions, firstly to respond acute stress, but secondly, and more generally, to promote change according to anticipated or current context, and to optimize physiological functioning for expected future conditions (Ellis, Del Giudice, & Shirtcliff, 2013). In line with this idea, researchers have emphasized the need to conceptualize biological stress regulation more broadly as *biologically sensitivity to context* (Boyce & Ellis, 2005), i.e., as functioning to regulate openness or susceptibility to environmental influences, especially those involving social interactions and support.

From this perspective, DHEA may therefore play a key role in the regulation of developmental plasticity, by integrating and "interpreting" environmental variation and adaptively shaping the development of the adolescent (Dufty, Clobert, & Møller, 2002; Ricklefs & Wikelski, 2002). This might especially be the case regarding adolescent changes in interpersonal relationships. Marceau et al. (2014) suggest that,

as they become more proficient at dealing with social situations, adolescents are able to recruit DHEA to buffer from the deleterious effects of higher cortisol. This would likely be evident in the basal functioning of the biological stress system, which calibrates its set-points with environmental demands and changes, thus indicating a level of physiological preparedness or anticipation of the individual's context (McEwen & Wingfield, 2003; Pruessner et al., 2010). We might, therefore, predict increases in basal DHEA to be associated with adolescent attachment, particularly those dimensions of the primary caregiver relationship that undergo change during this developmental period. In contrast to cortisol, however, there has been no investigation of basal, salivary DHEA and attachment in adolescence.

Learning how to manage the complexity of human social relationships is suggested to be one of the primary evolutionary functions of negotiating the transition from childhood to adolescence (Flinn & Ward, 2005). Whereas Bowlby's (1969) original formulation of Attachment Theory drew extensively on biological and ethological concepts, later theorists have tended to focus on the proximate level of explanation and have largely ignored the evolutionary underpinnings of attachment (Del Giudice, Angeleri, & Manera, 2009). The Adaptive Calibration Model (ACM; Del Giudice, Ellis, & Shirtcliff, 2011), an evolutionary-developmental theory, holds that these biological stress responses and patterns are modified by environmental circumstances around the time of puberty. Since research has demonstrated that physiological patterns of the biological stress system constitute a primary integrative pathway through which psychosocial environmental factors are transmuted into the behavioral manifestations (Del Giudice et al., 2011), associations between attachment behaviors and DHEA, in particular, might be expected.

But which attachment behaviors in particular would exhibit such associations? Particularly salient during adolescence is the process of separation–individuation from parents, which leads to increased autonomy and, therefore, less reliance on support from the primary caregiver relationship (Brown & Wright, 2001; Daniels, 1990; Noom, Deković, & Meeus, 1999). Research demonstrates decreasing levels of emotional intimacy and disclosure to parents and increasing closeness with peers across this period (Brown & Wright, 2001; Hamza & Willoughby, 2010). As stated, confiding is not only an attachment behavior currently important for policy drives, but also an important part of the mother-daughter relationship that may be associated with HPA axis regulation (Lyons-Ruth, Choi-Kain, Pechtel, Bertha, & Gunderson, 2011). Again, though, research investigating DHEA is much more limited; to our knowledge there is only one study of basal DHEA from which we might generate predictions for the current study: Heaney, Phillips and Carroll (2012) found that, in an elderly sample, individuals with less independence in carrying out activities of daily living displayed lower levels of DHEA in the morning period (Heaney et al., 2012). This supports the idea that our biological stress regulation system operates as an evolved mechanism that matches the individual's physiology and behavior to the environment (Ellis, Del Giudice, & Shirtcliff, 2013), which would point toward positive associations between DHEA and autonomy.

The Current Study

The current study sought to examine potential differential associations among attachment behaviors, basal levels of salivary cortisol and DHEA secretion, particularly in the period immediately post-awakening, in a group of healthy young females. We chose to investigate an all-female sample for reasons pertaining both to

attachment and to physiological development. There is evidence from evolutionary-developmental studies that attachment styles undergo a sex-specific reorganisation in middle childhood with insecurity of attachment style in females manifesting in more anxious strategies and males tending to adopt avoidant strategies (Del Giudice, 2009). From a neuroendocrine perspective, gender-specific differences regarding basal cortisol and DHEA activity and social relationships during adolescence are observed in several studies (Booth, Granger, & Shirtcliff, 2008; Marceau et al., 2014; Matchock, Dorn, & Susman, 2007).

The present methodology involved multiple saliva sampling over the day, in strict reference to time of awakening (thus including the CAR), on two consecutive sampling weekdays and with careful checks on participant adherence to protocol. An interview-based measure of current attachment style was used that included an assessment of specific indicators of attachment attitudes pertaining to avoidance and anxiety, as well as behaviors regarding the primary caregiver relationship, namely confiding and emotional support. We tested two main hypotheses. First, in line with previous studies of adults and suggesting links between dysregulated diurnal cortisol profiles and insecure-anxious attachment (Kidd, Hamer, & Steptoe, 2013; Oskis et al., 2011; Quirin et al., 2008), we expected specific dimensions of attachment anxiety, including fear of rejection and separation/abandonment, would predict a flatter awakening response of cortisol. Second, in line with evolutionary-developmental theories of biological stress regulation, we expected less attachment behavior/support seeking towards the primary caregiver, manifested by less confiding (presumably reflecting greater adolescent autonomy), would predict higher levels of basal DHEA.

Methods

Participants and Procedure

Fifty-five healthy females with an average age of 14.36 years (*SD* = 2.41) years took part in this study. All aspects of this study were approved by the local university ethics committee and conducted in accord with international standards for the protection of human subjects. A cross-sectional design was employed and volunteers were mostly recruited by approaching local schools. Individuals who expressed an interest were provided with an information flyer and a reply slip to complete personal details. Written parental permission was required for those under 16 years of age. On return of this reply slip, the researcher contacted the participant to arrange an appointment. Participants were briefed in detail on a one-to-one basis, usually in their home environment. After written informed consent was provided, each participant received a study pack, which included a copy of the participant information sheet, full standardized written instructions, saliva sampling materials and a self-report measure of anxiety. Participants' demographic and developmental information (see Table 1) was recorded and the Attachment Style Interview (ASI) was then carried out. All work undertaken in this study was conducted by the same researcher.

Participants were a relatively homogeneous population of female adolescents; most lived and attended public schools in the Greater London area and all were British born. The sample presented as healthy; all were non-smokers and were without acute or chronic illness; none took prescribed medication, including the oral contraceptive. All were screened for the presence of anxiety using the State-Trait Anxiety Inventory (STAI; Spielberger, 1970) and the mean state and trait anxiety scores (29.97 (*SD* = 6.78) and 34.27 (*SD* = 7.57), respectively) of the sample were

within age-appropriate normative range (see Wilson & Hayward (2006) for comparable data).

Measures

Attachment style. The Attachment Style Interview (ASI; (Bifulco et al., 2002) is an investigator-based interview that uses a contextualized, support-focused approach to assess current interpersonal behavior and attitudes. In this study, each participant's ASI took roughly one hour. The interview is semi-structured and falls naturally into two parts. In the first part, participants are asked questions to elicit specific examples of confiding and active emotional support in their primary caregiver (which was the mother for these participants). Next, the participant is assessed by attitudinal scales concerning avoidance/distance in relationships (mistrust, constraints on closeness, anger) or anxious attachment (fear of rejection, fear of separation, high desire for company). ASI scales are generally rated as "1: marked", "2: moderate", "3: some", "4: little/none," however, within the present analyses, the 1 to 4 codings were reversed to allow for more intuitive interpretations of findings. The ASI is a flexible measure and scores regarding caregiver support and attitudes can be combined to categorise overall attachment style in terms of the standard secure-anxious-avoidant typology. Alternatively, and in line with a more dimensional approach to the assessment of attachment, the separate ASI scales can be used to tease apart which specific dimensions relate to variables of interest (for example, see Oskis et al., 2013).

In terms of inter-rater reliability of the ASI, the scales are all of interval level; thus correlational analyses were carried out to determine inter-rater reliability. Thirty ASIs were blind-rated and for the attitudinal scales of mistrust, constraints on

closeness, fear of rejection, fear of separation and anger scales, *r* values ranged from .73 to .90. For confiding in mother and active emotional support from mother, *r* values were .89 and .83, respectively. All were significant at $p < .0005$. Previous work also demonstrated the ASI was internally consistent in large samples of healthy female adolescents and adults from both Europe and the USA (Bifulco et al., 2004). The ASI has convergent validity as demonstrated by its associations with self-esteem, childhood adversity and negative social context (Bifulco et al., 2002).

Salivary cortisol and DHEA. In the week following completion of the ASI, participants collected saliva samples on awakening (i.e., 0 minutes), 30 minutes and 12 hours post-awakening on two consecutive weekdays to capture secretory patterns relevant to both steroids, including the CAR. Profiles comprised of similar three sampling points provide a reliable assessment of HPA axis hormones in adolescents (for example Heaney et al., 2012; O'Connor et al., 2005). Saliva was collected by passively drooling through a straw into the small, appropriately-labelled plastic tube. For at least 30 minutes prior to the collection of each sample, participants had to adhere by guidelines of nil by mouth other than water and the avoidance of vigorous exercise and brushing teeth. Other than these requests, participants were free to follow their normal daily routine. Samples were stored in participants' home freezers on the same day of collection. Insulated packs were used to transfer samples to the laboratory where they were stored at -20°C until assay.

On each study day, participants recorded their method of waking up (whether naturally or by alarm clock), waking time and the exact times of collection of saliva samples. (It is noteworthy that including any of these situational variables as covariates in the analyses did not change the results presented here.) Each participant

was sent a text message at their individually-predicted awakening time. They were asked to reply stating both their actual awakening time and corresponding collection time of the awakening sample. If participants woke up earlier than their expected awakening time, they were asked to text the researcher with this same information. All participants made contact to provide information regarding awakening time and Sample 1 collection time. No participants woke up later than anticipated; therefore none were awoken by the text message. The information regarding awakening time allowed specific and personalized text message reminders to be sent to each individual over the course of the day to prompt each saliva sample collection.

For the assay process, samples were thawed and centrifuged at 1500 x g (3000 rpm) for 15 minutes. Cortisol concentration was determined by the Expanded Range High Sensitivity Enzyme Linked Immuno-Sorbent Assay developed by Salimetrics LLC (USA). Similarly, the Salimetrics Salivary DHEA Enzyme Immunoassay was used to measure DHEA concentrations. The DHEA assay was carried out prior to cortisol. Standard range for cortisol assay: .08 – 82.80 nmol/l. Correlation of assay with serum cortisol: $r = .91$, $p < .0005$, $n = 49$ samples. For the DHEA assay, the standard range was .03 – 3.47 nmol/l. Correlation of assay with serum DHEA: $r = .86$, $p < .0005$, $n = 39$ samples. For both cortisol and DHEA, intra and inter-assay variations were below 5% and 10%, respectively.

Analyses were conducted using the following outcome measures: the cortisol awakening response (CAR) was calculated as the 30 minute post-awakening sample minus the awakening sample (Edwards, Clow, Evans, & Hucklebridge, 2001; Heaney et al., 2012). For the CAR, greater scores reflect larger rises in the 30 minute post-awakening period. Smaller rises in the CAR have been linked to a range of poor physical and mental health outcomes (Kudielka & Kirschbaum, 2003; Shirtcliff &

Essex, 2008), hence our choice to analyze this index of cortisol secretion as opposed to mean levels. In contrast, DHEA does not exhibit a robust post-awakening dynamic, therefore, in line with previous investigations of DHEA (for example Hucklebridge, Hussain, Evans, & Clow, 2005; Matchock et al., 2007), mean levels were used. For both cortisol and DHEA, morning and evening levels were analyzed separately because of increasing evidence that these two periods of secretory activity might be subject to different regulatory influences (Edwards et al., 2001; Schmidt-Reinwald et al., 1999). The cortisol-DHEA ratio was calculated by dividing cortisol by DHEA secretion at each of the three time points. A high cortisol to DHEA ratio has been suggested to predispose adolescents to adverse psychological health outcomes (Goodyer, Herbert, & Altham, 1998; Goodyer et al., 1996; Goodyer, Herbert, Tamplin, & Altham, 2000). However, it remains unknown whether this is a consequence of high cortisol or low DHEA or both, which highlights the importance of investigating the secretion of both hormones separately, as well as in their ratio form.

Results

Correlates of Cortisol and DHEA

Raw cortisol and DHEA concentrations at each sampling point were moderately positively-skewed. A square-root transformation was applied, which reduced skewness statistics and normalized the data in line with Shapiro-Wilks' test All other study variables were normally distributed.

Pearson's correlations revealed that cortisol concentrations were significantly correlated over the two sampling days (0 minutes: $r = .55$, $p < .0005$; 30 minutes: $r = .47$, $p < .0005$; 12 hours post-awakening $r = .77$, $p < .0005$). This was also the case for DHEA levels (0 minutes: $r = .71$, $p < .0005$; 30 minutes: $r = .79$, $p < .0005$; 12

hours post-awakening: $r = .66$, $p < .0005$). Therefore, the mean concentration for each time point was calculated and used in all analyses.

Table 1 displays participants' mean (± SD) for measures of developmental, situational and neuroendocrine variables. Pearson's correlations were computed to see which specific dimensions of attachment style were related to cortisol and DHEA. The correlation matrix can be seen in Table 2. It is noteworthy that being post-menarche was associated with greater DHEA levels at all time points (0 minutes: $r = -.58$, $p < .0005$; 30 minutes: $r = -.46$, $p < .0005$; 12 hours post-awakening $r = -.38$, $p = .004$). Menarche status was also associated with the cortisol/DHEA ratio at awakening ($r = .490$, $p < .0005$) and 30 minutes post-awakening ($r = .46$, $p < .0005$). Given these findings, and that menarche status is known to affect cortisol and DHEA secretion (see (Matchock et al., 2007; A. Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2009)), this was partialled for in the correlations presented in Table 2.

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As expected the CAR exhibited significant associations with fear of rejection ($r = -.37$, $p = .01$) and fear of separation ($r = -.26$, $p = .05$), whereby those with a smaller rise in cortisol displayed higher levels of these anxious attachment characteristics. In contrast DHEA concentrations, at any time point, were not associated with anxious attachment attitudes. However, unlike for cortisol, greater DHEA concentrations at each time point were significantly associated with the attachment behavior of less confiding in mother (0 minutes: $r = -.42$, $p = .001$; 30

minutes: r = -.29, p = .03; 12 hours post-awakening: r = -.35, p = .01). Higher DHEA levels upon awakening were also correlated with less maternal active emotional support (r = -.32, p = .02).

The cortisol/DHEA ratio at awakening exhibited significant positive associations with anxious attachment characteristics of fear of rejection (r = .32, p = .02) and fear of separation (r = .38, p = .01). These findings were driven by levels of cortisol following awakening. The cortisol/DHEA ratio was not associated with any other feature of attachment. As stated, all results remained statistically significant when menarche status was partialled for in these correlations.

Predictors of Cortisol and DHEA

To identify which specific attachment style dimensions were independent predictors of cortisol and DHEA secretion and to examine the primary hypotheses of this study, multiple linear regression analyses were carried out using the individual attachment style interview scales that evidenced significant relationships in the correlational analyses above. Separate regression analyses were performed to predict 0min DHEA, the CAR and the 0min cortisol-DHEA ratio. To account for developmental status within the sample, menarche status was included in each regression. A similar approach to statistical analysis using linear multiple regression has been used in previous studies seeking to disentangle dimensions of attachment style (for example Meins, Harris-Waller, & Lloyd, 2008; Oskis et al., 2013)). A summary of the regression analyses can be seen in Table 3.

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For DHEA at 0 minutes post-awakening, the regression produced a significant model ($F_{(3, 51)} = 17.50$, $p = < .0005$) and menarche status and confiding in mother were identified as independent predictors; having experienced first menses and less confiding in mother both significantly predicted DHEA levels at awakening. The model predicting the CAR was also significant ($F_{(3, 51)} = 3.21$, $p = .03$), and fear of rejection, consistent with the zero-order correlations, was the only independent predictor, with more fear corresponded to a smaller rise post-awakening. For the cortisol-DHEA ratio at awakening, the model was significant too ($F_{(3, 51)} = 9.76$, $p = < .0005$) and fear of rejection was again a significant predictor, in addition to menarche status. This time though, being pre-menarche significantly predicted a greater cortisol-DHEA ratio at awakening, as did greater fear of separation.

Discussion

The significance of the biological stress system to the developmental understanding of adolescent attachment can be extended by investigating more than just the quintessential stress hormone cortisol. The largely understudied HPA axis hormone DHEA is fundamental to development and offers the opportunity to deepen our knowledge regarding the key attachment behavior of confiding, which is considered analogous to the trademark of the infant attachment system that is proximity-seeking (Bifulco et al., 2002; Freeman & Brown, 2001; Johnson et al., 2001). In the current study, we found that the hormones cortisol and DHEA are differentially associated with dimensions of attachment in female adolescents. As hypothesized, a smaller cortisol awakening response was predicted by greater fear of rejection. Fear of separation emerged as an independent predictor of the cortisol/DHEA ratio at awakening. In contrast, DHEA was not predicted by any

dimension of attachment anxiety. Instead, the attachment behavior of confiding in mother was a significant predictor of awakening DHEA levels, but not cortisol, with less confiding corresponding to greater DHEA levels.

The observed results were consistent with our theory-driven predictions, and the differential pattern of findings for cortisol and DHEA may suggest specificity of the aspects of care related to adolescent development. Cacioppo (1994) has argued that an individual's current pattern of neuroendocrine activity might be conceptualized as a function of his or her current, most important interpersonal relationships. Our findings appear to support this idea. To the extent that DHEA provides a putative index of progress in pubertal timing (Del Giudice et al., 2009), girls further along the adolescent developmental trajectory seem to be confiding in and depending less on their mothers. This also fits within developmental frameworks that place emphasis on the importance of autonomy for optimal development (Lee & Bell, 2003). As a psychophysiological biomarker, our results suggest that DHEA can be associated with age-appropriate changes in caregiver relationships and serves here to index an interpersonal shift in behavior. Adolescence is a unique time for attachment because in moving from attachment in infancy to attachment in adulthood mutuality is a key developmental task— i.e., the capacity to move flexibly between care-giving and care-seeking roles in the relationship (Clulow, 2007). Prior work suggests that mutuality in confiding, specifically within the mother-daughter relationship, may impact on HPA axis hormone regulation (Lyons-Ruth et al., 2011). This interpretation also fits given the role of DHEA in fertility status and the potential for the adolescent to now be a caregiver themselves. It is noteworthy that being post-menarche also significantly predicted DHEA levels at awakening, which is in line with the extensive literature showing the effect of developmental markers on DHEA

secretion (Matchock et al., 2007). Over-and-above this effect, we continued to observe that confiding behavior toward mothers was a significant independent predictor of awakening DHEA concentrations.

This observation is largely consistent with evolutionary-developmental theory, particularly the Adaptive Calibration Model (ACM; Del Giudice et al., 2011), which proposes that evolution may have led to modification of the stress system around the time of puberty. The current findings support this idea, indicating that during adolescence the HPA axis may need calibrating in order to deal with the demands of leaving the parental nest. One proposed means of calibration, as found in this study, is increased secretion of DHEA, which signals that the adolescent is developmentally "on schedule" to becoming reproductively mature (Del Giudice et al., 2009; Wolf & Kirschbaum, 1999). Adaptive scheduling of developmental tasks requires tight coordination between physiology and behavior (Del Giudice et al., 2011), so it is apt that as the female adolescent evolves from being a receiver of care from parents to being a potential caregiver themselves, less attachment behavior is directed towards the primary caregiver, in terms of confiding and, in Bowlby's (1969) original terms, using them as a safe haven. This is also consistent with the idea that adolescence affords an ''assessment period" in which children gauge their local environment and adjust their future developmental trajectories accordingly (Bogin, 1997).

Another possible interpretation regarding higher levels of DHEA, which is also relevant to our finding of a negative association between the CAR and fear of rejection, is that we are observing a puberty-related phenomenon whereby interactions with the parent cease to have the same potent buffering effect on the HPA axis that they did in infancy and childhood (Hostinar et al., 2014). In this study, the relationship between a smaller CAR and fear of rejection was hypothesized and in

line with previous studies (Oskis et al., 2011; Quirin et al., 2008; Tops et al., 2008). The hyperactivating nature of fearful and anxious attachment characteristics is likely to result in repeated elevations of cortisol (Mikulincer, Shaver, & Pereg, 2003). Therefore, flatness of the cortisol profile would be expected to develop over time as part of a necessary down-regulation in basal HPA axis activity, in order to avoid the detrimental effects of chronically elevated glucocorticoid levels (Adam, Sutton, Doane, & Mineka, 2008). These results also support the findings of a previous study, which linked attachment anxiety to reduced cell concentration in the left hippocampus −an area which is significant in the regulation of the CAR (Quirin et al., 2010). The knowledge that fears of rejection and abandonment/separation impact on health-relevant biological processes in adolescents provides specific targets to feed into practical applications of this work. This can be linked to, for example, peer-rejection interventions (see Boulton, 2013; Mikami, Lerner, & Lun, 2010), which have so far provided promising findings relating to adolescent health (Fröhlich, Pott, Albayrak, Hebebrand, & Pauli-Pott, 2011) and psychological disorders (Moretti, Odgers, Reppucci, & Catherine, 2011).

In the present study, fear of separation predicted a higher cortisol-DHEA ratio at awakening. The cortisol-DHEA has mostly been studied in relation to early-onset depression, where extensive findings point to a higher ratio in clinically depressed adolescents (Goodyer et al., 1998; Goodyer et al., 1996; Goodyer et al., 2000). However, it remains unknown whether that finding in the literature is due to either high cortisol or low DHEA or both: psychosocial factors may differentially affect cortisol or DHEA, which is not apparent by examination of the ratio alone (Goodyer, Park, Netherton, & Herbert, 2001). Our results suggest that high cortisol levels, and the link to anxious attachment, may play an important role in driving the cortisol-

DHEA ratio. In times of perceived threat or prolonged stress, similar to that experienced during fearful attachment states, steroid biosynthesis may shift from biosynthesis of adrenal androgens to glucocorticoid pathways (Lennartsson, Theorell, Kushnir, Bergquist, & Jonsdottir, 2013). Such a shift would lead to higher levels of cortisol and lower levels of DHEA, which would drive a higher cortisol-DHEA ratio. A further interpretation regarding the cortisol-DHEA ratio hypothesis is that DHEA balances cortisol and buffers the body from harmful effects of prolonged exposure to cortisol (for example Herbert, 1997; Kimonides, Spillantini, Sofroniew, Fawcett, & Herbert, 1999; Mao & Barger, 1998) so a high cortisol to DHEA ratio indicates an imbalance that, for example, may predispose adolescents to deleterious psychological health outcomes (Goodyer et al., 1998; Goodyer et al., 2003). This is also consistent with the accumulating literature that proposes a link between attachment anxiety, dysregulated cortisol and a range of poor physical and mental health outcomes (Fagundes et al., 2011; Jaremka et al., 2013; Puig et al., 2013). In a wider context, this also supports how psychophysiological research may serve as an important bridge to link prevailing psychosocial conditions to health (Sbarra & Coan, 2013).

Although this study is among the first investigations of basal DHEA and attachment, the cross-sectional nature of the work does not allow for the attribution of causality. The gold-standard for future work would be to adopt multi-method, longitudinal designs to track the diverse and many psychological, behavioral and physiological sequelae of the complex phenomenon that is human attachment. In addition, it would be useful for future studies to include a dimensional measure of pubertal development. This study also highlights the importance of conducting rigorous psychophysiological research. This is pertinent given recent findings suggesting that individuals may soon be able to measure their cortisol levels using just

their smartphones (see Baulkman, 2014), which raises significant issues for future work in this area of adolescent psychophysiology. Finally, our results also reveal the rich data that are to be gained from looking at specific, underlying constructs of attachment as opposed to simplified and general categories of secure vs. insecure, which unfortunately is still the case even in recent research on attachment (for example Neustadt, Chamorro-Premuzic, & Furnham, 2011).

Conclusion

The findings from this study demonstrate links between adolescent interpersonal processes and a biological system that is essential not only for stress regulation but also for developmental transitions. A sample of adolescent girls participated in the Attachment Style Interview, which provided a detailed assessment of their attachment attitudes concerning anxiety and avoidance, as well as confiding and emotional support from their maternal relationship. Diurnal patterns of two fundamental mediators of the biological stress system, cortisol and DHEA, were also assessed. Our findings revealed functionally different associations between cortisol and DHEA and dimensions of attachment in this adolescent sample. In support of previous research, cortisol secretion was predicted by attachment attitudes involving increased fear and anxiety. In contrast, increased DHEA secretion was only predicted by the objective attachment behavior of less confiding in mothers. These data correspond to previous research findings with females. Lyons-Ruth et al. (2011) found that confiding in mothers was *unrelated* cortisol activity. Given the differential physiological functions of these two hormones, it is therefore fitting that we found DHEA *was* related to confiding.

The pattern of cortisol tells us one story regarding the developmental understanding of adolescent attachment, but the pattern of DHEA tells another. Overall, our data indicate that these two HPA axis hormones are associated with their typical functions: cortisol for stress/anxiety and DHEA for age-related functions. Theoretically, therefore, our data support the normative shifts that occur in attachment to caregivers around adolescence, and that studying these shifts can be informed by evolutionary-developmental theory. Practically, we suggest that knowing that attachment fears of rejection and separation, as well as confiding in mother, impact on physiological functioning could provide clear targets for interventions seeking to improve adolescent health and well-being.

The manuscript does not contain clinical studies or patient data.

There is no conflict of interest.

References

Adam, E. K., Sutton, J. M., Doane, L. D., & Mineka, S. (2008). Incorporating hypothalamic-pituitary-adrenal axis measures into preventive interventions for adolescent depression: Are we there yet? *Development and Psychopathology, 20*(3), 975-1001.

Allen, J. P., & Land, D. (1999). Attachment in adolescence. In J. Cassidy & P. R. Shaver (Eds.), *Handbook of attachment: Theory, research and clinical applications* (pp. 319-335). New York: Guildford Press.

Angold, A. (2003). Adolescent depression, cortisol and DHEA. *Psychological Medicine, 33*(4), 573-581.

Baulkman, J. (2014). *Cortisol Testing May Soon Be Available On All Smartphones* http://www.universityherald.com/articles/10095/20140624/cortisol-testing-may-soon-be-available-on-all-smartphones.htm. Accessed 25 June 2014

Barnhart, K. T., Freeman, E., Grisso, J. A., Rader, D. J., Sammel, M., Kapoor, S., et al. (1999). The effect of deydroepiandrosterone supplementation to symptomatic perimenopausal women on serum endocrine profiles, lipid parameters, and health-related quality of life. *Journal of Clinical Endocrinology and Metabolism, 84*(11), 3896-3902.

Bifulco, A., Figueiredo, B., Guedeney, N., Gorman, L. L., Hayes, S., Muzik, M., et al. (2004). Maternal attachment style and depression associated with childbirth: Preliminary results from a European and US cross-cultural study. *British Journal of Psychiatry, 184*, S31-S37.

Bifulco, A., Moran, P., Jacobs, C., & Bunn, A. (2009). Problem partners and parenting: Exploring linkages with maternal insecure attachment style and adolescent offspring internalizing disorder. *Attachment & Human Development, 11*(1), 69-85.

Bifulco, A., Moran, P. M., Ball, C., & Bernazzani, O. (2002). Adult attachment style, I: Its relationship to clinical depression. *Social Psychiatry and Psychiatric Epidemiology, 37*(2), 50-59.

Bogin, B. (1997). Evolutionary hypotheses for human childhood. *American Journal of Physical Anthropology, 104*(S25), 63-89.

Booth, A., Granger, D. A., & Shirtcliff, E. A. (2008). Gender- • and Age-Related Differences in the Association Between Social Relationship Quality and Trait

Levels of Salivary Cortisol. *Journal of Research on Adolescence, 18*(2), 239-260.

Boulton, M. J. (2013). The effects of victim of bullying reputation on adolescents' choice of friends: Mediation by fear of becoming a victim of bullying, moderation by victim status, and implications for befriending interventions. *Journal of Experimental Child Psychology, 114*(1), 146-160.

Bowlby, J. (1969). *Attachment and loss. Vol. 1: Attachment*.

Boyce, W. T., & Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionaryâ€“developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology, 17*(02), 271-301.

Brown, L. S., & Wright, J. (2001). Attachment theory in adolescence and its relevance to developmental psychopathology. *Clinical Psychology & Psychotherapy, 8*(1), 15-32.

Buvat, J. (2003). Androgen therapy with dehydroepiandrosterone. *World Journal of Urology, 21*(5), 346-355.

Cacioppo, J. T. (1994). Social neuroscience: Autonomic, neuroendocrine, and immune responses to stress. *Psychophysiology, 31*(2), 113-128.

Clulow, C. (2007). John Bowlby and couple psychotherapy. *Attachment & Human Development, 9*(4), 343-353.

Daniels, J. A. (1990). Adolescent Separation-Individuation and Family Transitions. *Adolescence, 25*(97), 105-116.

Del Giudice, M. (2009). Sex, attachment, and the development of reproductive strategies. *Behavioral and Brain Sciences, 32*(01), 1-21.

Del Giudice, M., Angeleri, R., & Manera, V. (2009). The juvenile transition: A developmental switch point in human life history. *Developmental Review, 29*(1), 1-31.

Del Giudice, M., Ellis, B. J., & Shirtcliff, E. A. (2011). The adaptive calibration model of stress responsivity. *Neuroscience & Biobehavioral Reviews, 35*(7), 1562-1592.

Department for Education and Skills, Preparing and Assessing Prospective Adopters, www.every childmatters.gov.uk/ resources-and-practice/IG00138/, 2006. Accessed 25 June 2014.

Diamond, L. M. (2001). Contributions of psychophysiology to research on adult attachment: Review and recommendations. *Personality and Social Psychology Review, 5*(4), 276-295.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130*(3), 355-391.

Dufty, A. M., Clobert, J., & Møller, A. P. (2002). Hormones, developmental plasticity and adaptation. *Trends in Ecology & Evolution, 17*(4), 190-196.

Edwards, S., Clow, A., Evans, P., & Hucklebridge, F. (2001). Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life Sciences, 68*(18), 2093-2103.

Ellis, B. J., Del Giudice, M., & Shirtcliff, E. A. (Eds.). (2013). *Beyond allostatic load: The stress response system as a mechanism of conditional adaptation* (2 ed.). New York: Wiley.

Erikson, E. H. (1968). *Identity: Youth and crisis*: WW Norton & Company.

Fagundes, C. P., Bennett, J. M., Derry, H. M., & Kiecolt-Glaser, J. K. (2011). Relationships and Inflammation across the Lifespan: Social Developmental Pathways to Disease. *Social and Personality Psychology Compass, 5*(11), 891-903.

Flinn, M. V., & Ward, C. V. (2005). Ontogeny and evolution of the social child. *Origins of the social mind: Evolutionary psychology and child development, ed. BJ Ellis & DF Bjorklund*, 19-44.

Flynn, M. A., Weaver-Osterholtz, D., Sharpe-Timms, K. L., Allen, S., & Krause, G. (1999). Dehydroepiandrosterone replacement in aging humans. *Journal of Clinical Endocrinology and Metabolism, 84*(5), 1527-1533.

Freeman, H., & Brown, B. B. (2001). Primary attachment to parents and peers during adolescence: Differences by attachment style. *Journal of Youth and Adolescence, 30*(6), 653-674.

Fröhlich, G., Pott, W., Albayrak, A., Hebebrand, J., & Pauli-Pott, U. (2011). Conditions of long-term success in a lifestyle intervention for overweight and obese youths. *Pediatrics, 128*(4), e779-e785.

Goodyer, I. M., Herbert, J., & Altham, P. M. E. (1998). Adrenal steroid secretion and major depression in 8- to 16- year-olds, III. Influence of cortisol/DHEA ratio at presentation on subsequent rates of disappointing life events and persistent major depression. *Psychological Medicine, 28*(2), 265-273.

Goodyer, I. M., Herbert, J., Altham, P. M. E., Pearson, J., Secher, S. M., & Shiers, H. M. (1996). Adrenal secretion during major depression in 8- to 16-year-olds, I. Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. *Psychological Medicine, 26*(2), 245-256.

Goodyer, I. M., Herbert, J., & Tamplin, A. (2003). Psychoendocrine antecedents of persistent first-episode major depression in adolescents: a community-based longitudinal enquiry. *Psychological Medicine, 33*(4), 601-610.

Goodyer, I. M., Herbert, J., Tamplin, A., & Altham, P. M. E. (2000). Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *British Journal of Psychiatry, 177*, 499-504.

Goodyer, I. M., Park, R. J., Netherton, C. M., & Herbert, J. (2001). Possible role of cortisol and dehydroepiandrosterone in human development and psychopathology. *British Journal of Psychiatry, 179*, 243-249.

Guttmann-Steinmetz, S., & Crowell, J. A. (2006). Attachment and externalizing disorders: A developmental psychopathology perspective. *Journal of the American Academy of Child & Adolescent Psychiatry*, *45*(4), 440-451.

Hamza, C. A., & Willoughby, T. (2010). Perceived parental monitoring, adolescent disclosure, and adolescent depressive symptoms: A longitudinal examination. *Journal of Youth and Adolescence, 40*(7), 902-915.

Heaney, J. L. J., Phillips, A. C., & Carroll, D. (2012). Ageing, physical function, and the diurnal rhythms of cortisol and dehydroepiandrosterone. *Psychoneuroendocrinology, 37*(3), 341-349.

Herbert, J. (1997). Fortnighly review. Stress, the brain, and mental illness. *BMJ: British Medical Journal*, *315*(7107), 530.

Hicks, A. M., & Diamond, L. M. (2011). Don't go to bed angry: Attachment, conflict, and affective and physiological reactivity. *Personal Relationships, 18*(2), 266-284.

Hostinar, C. E., Sullivan, R. M., & Gunnar, M. R. (2014). Psychobiological mechanisms underlying the social buffering of the

hypothalamicâ€“pituitaryâ€“adrenocortical axis: A review of animal models and human studies across development. *Psychological Bulletin, 140*(1), 256-282.

Hucklebridge, F., Hussain, T., Evans, P., & Clow, A. (2005). The diurnal patterns of the adrenal steroids cortisol and dehydroepiandrosterone (DHEA) in relation to awakening. *Psychoneuroendocrinology, 30*(1), 51-57.

Jaremka, L. M., Glaser, R., Loving, T. J., Malarkey, W. B., Stowell, J. R., & Kiecolt-Glaser, J. K. (2013). Attachment anxiety is linked to alterations in cortisol production and cellular immunity. *Psychological Science*, 0956797612452571.

Johnson, M. D., Bebb, R. A., & Sirrs, S. M. (2002). Uses of DHEA in aging and other disease states. *Ageing Research Reviews, 1*(1), 29-41.

Johnson, S. M., Makinen, J. A., & Millikin, J. W. (2001). Attachment injuries in couple relationships: A new perspective on impasses in couples therapy. *Journal of Marital and Family Therapy, 27*(2), 145-155.

Kidd, T., Hamer, M., & Steptoe, A. (2013). Adult attachment style and cortisol responses across the day in older adults. *Psychophysiology, 50*(9), 841-847.

Kimonides, V. G., Spillantini, M. G., Sofroniew, M. V., Fawcett, J. W., & Herbert, J. (1999). Dehydroepiandrosterone antagonizes the neurotoxic effects of corticosterone and translocation of stress-activated protein kinase 3 in hippocampal primary cultures. *Neuroscience, 89*(2), 429-436.

Kroboth, P. D., Salek, F. S., Pittenger, A. L., Fabian, T. J., & Frye, R. F. (1999). DHEA and DHEA-S: A review. *Journal of Clinical Pharmacology, 39*(4), 327-348.

Kudielka, B. M., & Kirschbaum, C. (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology, 28*(1), 35-47.

Labrie, F., Belanger, A., Cusan, L., & Candas, B. (1997). Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: Intracrinology. *Journal of Clinical Endocrinology and Metabolism, 82*(8), 2403-2409.

Lee, J. M., & Bell, N. J. (2003). Individual differences in attachment–autonomy configurations: linkages with substance use and youth competencies. *Journal of Adolescence, 26*(3), 347-361.

Lennartsson, A.-K., Theorell, T. r., Kushnir, M. M., Bergquist, J., & Jonsdottir, I. r. H. (2013). Perceived stress at work is associated with attenuated DHEA-S response during acute psychosocial stress. *Psychoneuroendocrinology, 38*(9), 1650-1657.

Lyons-Ruth, K., Choi-Kain, L., Pechtel, P., Bertha, E., & Gunderson, J. (2011). Perceived parental protection and cortisol responses among young females with borderline personality disorder and controls. *Psychiatry Research, 189*(3), 426-432.

Mao, X., & Barger, S. W. (1998). Neuroprotection by dehydroepiandrosterone sulfate: role of an NFκB-like factor. *Neuroreport, 9*(4), 759-763.

Marceau, K., Shirtcliff, E. A., Hastings, P. D., Klimes-Dougan, B., Zahn-Waxler, C., Dorn, L. D., et al. (2014). Within-adolescent coupled changes in cortisol with DHEA and testosterone in response to three stressors during adolescence. *Psychoneuroendocrinology, 41*, 33-45.

Matchock, R. L., Dorn, L. D., & Susman, E. J. (2007). Diurnal and seasonal cortisol, testosterone and DHEA rhythms in boys and girls during puberty *Chronobiology International, 24*(5), 969-990.

Maxwell, M., & Cook, L. (2014). The portrayal of the adopted child in British newspapers and magazines. *Vulnerable Children and Youth Studies*, 1-5.

Milan, S., Zona, K., & Snow, S. (2013). Pathways to Adolescent Internalizing: Early Attachment Insecurity as a Lasting Source of Vulnerability. *Journal of Clinical Child & Adolescent Psychology*, 42(3), 371-383.

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior, 43*(1), 2-15.

Meins, E., Harris-Waller, J., & Lloyd, A. (2008). Understanding alexithymia: Associations with peer attachment style and mind-mindedness. *Personality and Individual Differences, 45*(2), 146-152.

Mikami, A. Y., Lerner, M. D., & Lun, J. (2010). Social context influences on children's rejection by their peers. *Child Development Perspectives, 4*(2), 123-130.

Mikulincer, M., Shaver, P. R., & Pereg, D. (2003). Attachment theory and affect regulation: The dynamics, development, and cognitive consequences of attachment-related strategies. *Motivation and Emotion, 27*(2), 77-102.

Morales, A. J., Nolan, J. J., Nelson, J. C., & Yen, S. S. C. (1994). Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. *Journal of Clinical Endocrinology and Metabolism, 78*(6), 1360-1367.

Moretti, M. M., Odgers, C., Reppucci, N. D., & Catherine, N. L. A. (2011). Serious conduct problems among girls at risk: Translating research into intervention. *International Journal of Child, Youth and Family Studies, 2*(1/2), 142-161.

Moretti, M. M., & Peled, M. (2004). Adolescent-parent attachment: Bonds that support healthy development. *Paediatrics & child health, 9*(8), 551.

Murray-Close, D. (2013), Psychophysiology of Adolescent Peer Relations I: Theory and Research Findings. *Journal of Research on Adolescence, 23*, 236–259.

Netherton, C., Goodyer, I., Tamplin, A., & Herbert, J. (2004). Salivary cortisol and dehydroepiandrosterone in relation to puberty and gender. *Psychoneuroendocrinology, 29*(2), 125-140.

Neustadt, E. A., Chamorro-Premuzic, T., & Furnham, A. (2011). Attachment at work and performance. *Attachment & Human Development, 13*(5), 471-488.

Noom, M. J., Deković, M., & Meeus, W. H. (1999). Autonomy, attachment and psychosocial adjustment during adolescence: a double-edged sword? *Journal of Adolescence, 22*(6), 771-783.

O'Connor, T. G., Ben-Shlomo, Y., Heron, J., Golding, J., Adams, D., & Glover, V. (2005). Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. *Biological Psychiatry, 58*(3), 211-217.

Oskis, A., Clow, A., Hucklebridge, F., Bifulco, A., Jacobs, C., & Loveday, C. (2013). Understanding alexithymia in female adolescents: The role of attachment style. *Personality and Individual Differences, 54*(1), 97-102.

Oskis, A., Loveday, C., Hucklebridge, F., Thorn, L., & Clow, A. (2009). Diurnal patterns of salivary cortisol across the adolescent period in healthy females. *Psychoneuroendocrinology, 34*(3), 307-316.

Oskis, A., Loveday, C., Hucklebridge, F., Thorn, L., & Clow, A. (2011). Anxious attachment style and salivary cortisol dysregulation in healthy female children and adolescents. *Journal of Child Psychology and Psychiatry, 52*(2), 111-118.

Pruessner, J. C., Dedovic, K., Pruessner, M., Lord, C., Buss, C., Collins, L., et al. (2010). Stress regulation in the central nervous system: evidence from structural and functional neuroimaging studies in human populations. *Psychoneuroendocrinology, 35*(1), 179-191.

Puig, J., Englund, M. M., Simpson, J. A., & Collins, W. A. (2013). Predicting adult physical illness from infant attachment: A prospective longitudinal study. *Health Psychology, 32*(4), 409-417.

Quirin, M., Gillath, O., Pruessner, J. C., & Eggert, L. D. (2010). Adult attachment insecurity and hippocampal cell density. *Social Cognitive and Affective Neuroscience, 5*(1), 39-47.

Quirin, M., Pruessner, J. C., & Kuhl, J. (2008). HPA system regulation and adult attachment anxiety: Individual differences in reactive and awakening cortisol. *Psychoneuroendocrinology, 33*(5), 581-590.

Ricklefs, R. E., & Wikelski, M. (2002). The physiology/life-history nexus. *Trends in Ecology & Evolution, 17*(10), 462-468.

Rifkin-Graboi, A. (2008). Attachment status and salivary cortisol in a normal day and during simulated interpersonal stress in young men. *Stress: The International Journal on the Biology of Stress, 11*(3), 210-224.

Rosenthal, N. L. & Kobak, R. (2010), Assessing Adolescents' Attachment Hierarchies: Differences Across Developmental Periods and Associations With Individual Adaptation. *Journal of Research on Adolescence, 20,* 678–706.

Sbarra, D. A., & Hazan, C. (2008). Coregulation, dysregulation, self-regulation: An integrative analysis and empirical agenda for understanding adult attachment, separation, loss, and recovery. *Personality and Social Psychology Review, 12*(2), 141-167.

Sbarra, D. A., & Coan, J. A. (2013). Theory, method, and prediction in the psychophysiology of relationships. *International Journal of Psychophysiology,88*(3), 219-223.

Schmidt-Reinwald, A., Pruessner, J. C., Hellhammer, D. H., Federenko, I., Rohleder, N., Schürmeyer, T. H., et al. (1999). The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sciences, 64*(18), 1653-1660.

Scott, S., Briskman, J., Woolgar, M., Humayun, S., & O'Connor, T. G. (2011). Attachment in adolescence: overlap with parenting and unique prediction of behavioural adjustment. *Journal of Child Psychology and Psychiatry, 52*(10), 1052-1062.

Shirtcliff, E. A., & Essex, M. J. (2008). Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Developmental Psychobiology, 50*(7), 690-703.

Shirtcliff, E. A., Zahn-Waxler, C., Klimes-Dougan, B., & Slattery, M. (2007). Salivary dehydroepiandrosterone responsiveness to social challenge in adolescents with internalizing problems. *Journal of Child Psychology and Psychiatry, 48*(6), 580-591.

Spielberger, C. D. (1970). *Manual for the State-Trait Anxiety Inventory (Self-Evaluation Questionnaire)*. California: Consulting Psychologists Press.

Sroufe, L. A. (2005). Attachment and development: A prospective, longitudinal study from birth to adulthood. *Attachment & Human Development, 7*(4), 349-367.

Tops, M., Riese, H., Oldehinkel, A. J., Rijsdijk, F. V., & Ormel, J. (2008). Rejection sensitivity relates to hypocortisolism and depressed mood state in young women. *Psychoneuroendocrinology, 33*(5), 551-559.

Wemm, S., Koone, T., Blough, E. R., Mewaldt, S., & Bardi, M. (2010). The role of DHEA in relation to problem solving and academic performance. *Biological Psychology, 85*(1), 53-61.

Wilson, K. A., & Hayward, C. (2006). Unique contributions of anxiety sensitivity to avoidance: A prospective study in adolescents. *Behaviour Research and Therapy, 44*(4), 601-609.

Wolf, O. T., & Kirschbaum, C. (1999). Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. *Brain Research Reviews, 30*(3), 264-288.

Wolf, O. T., Kudielka, B. M., Hellhammer, D. H., Hellhammer, J., & Kirschbaum, C. (1998). Opposing effects of DHEA replacement in elderly subjects on declarative memory and attention after exposure to a laboratory stressor. *Psychoneuroendocrinology, 23*(6), 617-629.

Wolf, O. T., Neumann, O., Hellhammer, D. H., Geiben, A. C., Strasburger, C. J., Dressendorfer, R. A., Pirke, K.M., & Kirschbaum, C. (1997). Effects of a two-week physiological dehydroepiandrosterone substitution on cognitive performance and well-being in healthy elderly women and men. *Journal of Clinical Endocrinology and Metabolism, 82*(7), 2363-2367.

UNICEF. (2007). *Child poverty in perspective: An overview of child well-being in rich countries* (No. inreca07/19). UNICEF Innocenti Research Centre.