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The Developmental Relationship between DHEA and Visual Attention Is Mediated by Structural Plasticity of Cortico-Amygdalar Networks

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Abstract

Humans and the great apes are the only species demonstrated to exhibit adrenarche, a key developmental event leading to increased production of dehydroepiandrosterone (DHEA), suggesting that this hormone may play an important evolutionary role. Similarly, visual attention networks have been shown to evolve in a human-specific manner, with some anatomical connections and elements of cortical organization exclusive to our species. Existing studies of human brain development support the notion that DHEA shows significant uptake in cortical structures and the amygdala, and as such, could be involved in the bottom-up regulation of visual attention. Here we examined associations between DHEA, structural covariance of the amygdala with whole-brain cortical thickness, and tests of visual attention, in a longitudinal sample of

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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typically developing children and adolescents 6 to 22 years of age. We found that DHEA predicted covariance between amygdalar volume and the left occipital pole, right somatosensory parietal cortex and right anterior cingulate cortex. Amygdala-occipital covariance predicted visual awareness; amygdala-parietal covariance predicted visuo-motor dexterity and processing speed; amygdala-prefrontal covariance predicted generalized attentional impairment. Further, effects of DHEA were above and beyond those of age and sex, as well as distinct from those of pubertal stage, estradiol and testosterone. These findings support the notion that DHEA may play a unique role in shaping amygdala-dependent cortical plasticity and in regulating ‘bottom-up’ visual attention processes from childhood to young adulthood.

Keywords

Adrenarche; Androgen; Visual Awareness; Visuo-Motor Dexterity; Adolescents; Human Brain

1. INTRODUCTION

Humans and the great apes are the only species demonstrated to exhibit adrenarche, a key developmental event leading to increased production of dehydroepiandrosterone (DHEA) (Remer et al., 2005), suggesting that this hormone may play an important evolutionary role. DHEA levels represent the most abundant steroid hormone in production across the lifespan, maintained at high levels from middle childhood until the third decade of life (Adams, 1985).

Evidence at the molecular level has confirmed the important role of DHEA in enhancing neuronal and glial plasticity, through a variety of peripheral and central mechanisms (Compagnone and Mellon, 2000; Li et al., 2008; Maninger et al., 2009). Of these, the most relevant to human development may be the anti-glucocorticoid effects of DHEA, which play an important role in metabolically active brain regions. Through these anti-glucocorticoid effects, DHEA preserves anabolic potential by increasing reserves in mitochondrial energy (Campbell, 2011). Because release of neurotransmitters during neuronal firing is thought to rely directly on mitochondrial energy, these effects of DHEA represent a direct mechanism through which it could alter activity-dependent synaptogenesis (Vos et al., 2010).

Similar to molecular studies, existing studies of human brain development support the notion that DHEA may be involved in preserving cortical plasticity in brain networks involved in cognitive control, such as the left dorsolateral prefrontal cortex, right temporoparietal junction, right premotor and right entorhinal cortex (Herting et al., 2015; Nguyen et al., 2013b). In addition, DHEA administration has been specifically linked to optimal performance on cognitive functions such as attention and executive control, with paradoxical detrimental effects on declarative memory (Sripada et al., 2013; Strous et al., 2001; Wolf et al., 1998).

Yet, beyond cortical structures, DHEA also shows significant uptake in the amygdala (Regelson and Kalimi, 1994; Sripada et al., 2013), a structure traditionally thought to be the affective center of the brain (Sah et al., 2003). Interestingly, there is accumulating evidence to suggest that the amygdala is also centrally involved in the ‘bottom-up’ regulation of

primary (i.e. perceptual salience) and secondary (i.e. goal-directed) attentional processes (Pessoa, 2008). The relationship between primary and secondary attentional processes may be facilitated by amygdala-dependent cortical plasticity, a process through which activation of the amygdala induces plasticity in sensory cortical regions in a modality-specific manner (Phelps and LeDoux, 2005). These structural effects of the amygdala are supported by previous findings of anatomical covariance between the amygdala and bilateral dorsolateral and dorsomedial prefrontal, inferior parietal, as well as bilateral orbital and ventromedial prefrontal cortices (Albaugh et al., 2013).

‘Bottom up’ cortical-subcortical systems have been shown to mature earlier than ‘top-down’ systems during adolescence (Casey and Jones, 2010). Of particular interest is the existence of an afferent subcortical visual pathway directly connected to the amygdala, with efferent projections from the amygdala to sensory cortical regions subsequently involved in ‘bottom-up’ regulation of visual awareness (Morris et al., 2001; Pasley et al., 2004; Pegna et al., 2005; Vuilleumier et al., 2003; Williams et al., 2004). Amygdala-dependent regulation of visual awareness may extend to the control of simple motor and more complex behaviors in response to visual stimuli (Pessoa, 2008; Phelps and LeDoux, 2005). These ‘bottom-up’ visual attention networks emerge during middle childhood, coinciding with increased DHEA production (Campbell, 2011). Similar to the unique presence of adrenarche in humans and the great apes, visual attention networks have been shown to evolve in a human-specific manner, with some anatomical connections and elements of cortical organization exclusive to our species (Patel et al., 2015; Preuss and Coleman, 2002). Taken together, the timing of the abrupt rise of DHEA, its effects on cortico-amygdalar structures and cognitive function, and its importance in human development all point toward a potentially unique role in the ‘bottom-up’ regulation of amygdalar-dependent cortical plasticity and visual attention in humans.

In sum, DHEA may regulate the relationship, or covariance, between amygdalar and cortical structures. DHEA-related structural covariance may in turn parallel the development of functional networks (Raznahan et al., 2011), leading to altered performance on measures of visual attention. To test these hypotheses, we examined associations between DHEA, structural covariance of the amygdala with whole-brain cortical thickness, and tests of visual attention, in a longitudinal sample of typically developing children and adolescents 6 to 22 years of age.

2. METHODS AND MATERIALS

2.1 Sampling and Recruitment

The National Institutes of Health (NIH) MRI Study of Normal Brain Development is a multi-site project that aimed to provide a normative database to characterize healthy brain maturation. Subjects were recruited across the United States with a population-based sampling method seeking to achieve a representative sample in terms of income level, race and ethnicity (Evans, 2006). All experiments on human subjects were conducted in accordance with the Declaration of Helsinki. All procedures were carried out with the adequate understanding and written parental consent, as well as assent of the subjects (or consent, if ≥ 18 years old). Subjects underwent repeated magnetic resonance brain imaging

(MRI) every 2 years, with a maximum of 3 scans over 4 years. The sample was limited to developmentally healthy children with rigorous exclusion criteria, described in detail elsewhere (Evans, 2006). In particular, any children with a current or past treatment for language disorder (simple articulation disorders not exclusionary); and a lifetime history of Axis I psychiatric disorder (except for simple phobia, social phobia, adjustment disorder, oppositional defiant disorder, enuresis, encopresis, nicotine dependency)' were excluded from the study. After strict quality control of MRI data (see section 2.2) and the exclusion of scans without hormonal measurements or behavioral parameters, 216 subjects were used for hormonal-related analyses (324 scans) and 192 to 210 subjects (261 to 324 scans) for cognitive analyses, depending on data available for each cognitive test (see Table 1 for more details).

2.2 Neuroimaging Measures

A three-dimensional T1-weighted (T1W) Spoiled Gradient Recalled (SPGR) echo sequence from 1.5 Tesla scanners was obtained on each participant, with 1mm isotropic data acquired sagittally from the entire head for most scanners. In addition, T2-weighted (T2W) and proton density-weighted (PDW) images were acquired using a two-dimensional (2D) multi-slice (2mm) dual echo fast spin echo (FSE) sequence.

Fully automated analysis of whole-brain cortical thickness was done through the CIVET pipeline, developed at the Montreal Neurological Institute (MNI). First, a multistage quality control process was implemented, as described previously (Nguyen et al., 2013a; Nguyen et al., 2013b), excluding subjects with white or gray matter artifacts. All quality-controlled MR images were subsequently processed through the CIVET pipeline. These processing steps have been described at length in other publications (Nguyen et al., 2013a; Nguyen et al., 2013b).

Volumetric measures of the amygdala were obtained from MRI data using a fully automated segmentation method validated in human subjects (Collins and Pruessner, 2010). This method utilizes a large, manually labeled MRI dataset ($n = 80$) of young healthy adults that serves as a template library (Pruessner et al., 2001). The manual segmentation was done by four different raters, and intra-class intra-rater and inter-rater reliability varied between $r=0.83$ for the right and $r=0.95$ for the left amygdala (Pruessner et al., 2000). From this manual segmentation, a fully automated method was derived, characterized by label fusion techniques that combine segmentations from a subset of 'n' most similar templates. Specifically, each template is used to produce an independent segmentation of the subject using the ANIMAL pipeline (Collins and Evans, 1997), followed by a thresholding step to eliminate cerebrospinal fluid, which results in 'n' different segmentations. To fuse the segmentations at each voxel, a voting strategy is used; the label with the most votes from the 'n' templates is assigned to the voxel. Combining multiple segmentations minimizes errors and maximizes consistency between segmentations. When using $n = 11$ templates, the label fusion technique has been shown to yield an optimal median Dice Kappa of 0.826 and Jaccard similarity of 0.703 for the amygdala (Collins and Pruessner, 2010).

2.3 Hormonal and Pubertal Measures

During each MRI visit, children provided two separate 1–3 cm³ samples of saliva, collected on the day of the scan, which were assayed by enzyme-linked immunosorbent assay (ELISA) methods, and the average results used as a measure of hormonal levels. The intra-assay and inter-assay coefficients of variation (COVs) were 6.5% and 16.2% for DHEA, 6.1% and 13.5% for testosterone, and 4.1% and 9.1% for estradiol, respectively (Salimetrics ELISA, State College, PA; Salimetrics Salivary ELISA Kit, State College, PA). At the next MRI, a similar procedure was followed and the child again provided two separate saliva samples for hormonal measurement.

Salivary sampling measures the free, non-protein bound, biologically active portions of circulating hormonal levels relevant to studies of brain-hormone associations (Khan-Dawood et al., 1984; Worthman et al., 1990). DHEA, as opposed to its sulfated hydrophilic form DHEAS, is easily measured in saliva and crosses the blood-brain barrier due to its lipophilicity (Stanczyk, 2006; Vining and McGinley, 1987). In contrast to the reported local CNS production of DHEA(S) in the rat brain, de novo synthesis of DHEA in the human brain is unlikely to be significant given the low/negligible levels of P450c17 and cytochrome b5 in the human CNS (Baulieu, 1998; Compagnone and Mellon, 2000). Should local CNS production of DHEA still proceed, it would nonetheless be dwarfed by the high net influx of peripheral DHEA into the CNS, the major portion of which is produced in the adrenal cortex (Compagnone and Mellon, 2000; Maninger et al., 2009). Further evidence supporting this model of DHEA synthesis comes from a study of human subjects in which serum DHEA levels were shown to correlate with cerebrospinal fluid DHEA levels (Kancheva et al., 2011). Therefore, peripheral DHEA levels may be especially representative of CNS levels and relevant to the study of brain developmental changes (Compagnone and Mellon, 2000).

Androgen levels, including those of DHEA, have been shown to follow diurnal and seasonal patterns in response to the pulsatile release of adrenocorticotrophic hormone and gonadotropin-releasing hormone, particularly in boys (Brambilla et al., 2009; Stanczyk, 2006). To control for this, we have included collection time, sex and season as covariates in hormonal-related analyses (see section 2.5). Finally, to measure pubertal maturation, the Pubertal Development Scale (PDS) was administered by a physician to all subjects included in this study (Petersen et al., 1988). This scale has been shown to have good reliability (coefficient alpha: 0.77) and validity ($r^2=0.61-0.67$) compared to physical examination (Petersen et al., 1988). During an interview with the child/adolescent, questions were asked about physical development. We computed a puberty variable consisting of 5 stages, representing increasing levels of physical maturity similar to Tanner staging, previously described (Nguyen et al., 2013a).

2.4 Cognitive Measures

Both DHEA administration and endogenous DHEA levels have been associated with improved cognition in men and women, including higher levels of attention, concentration, and visuo-spatial abilities (Davis et al., 2008; Fonda et al., 2005; van Niekerk et al., 2001; Wolf et al., 1998). However, some studies also document a DHEA-related decrease in post-stress recall of visual material and conjunctive emotional memory, perhaps related to

decreased salience of emotional stimuli (Sripada et al., 2013; Wolf et al., 1998). Therefore, we selected measures with limited emphasis on delayed recall and short-term memory, including: (1) ‘primary’ dimensions of awareness, persistence, and processing speed for visual stimuli; and (2) ‘secondary’, more global measures of behavioral and attentional control.

‘Primary’ attentional processes, as used here, refer to the perceptual salience of sensory stimuli, presumably through reciprocal connections between the amygdala and sensory cortex (Phelps and LeDoux, 2005). To test primary dimensions of visual attention, we selected the Woodcock-Johnson (3rd version; WJ-III) Letter-Word Identification subtest, which features visual detection and analysis for individual letters, and recognition of visual word forms. i.e. visual awareness. Words may or may not be familiar, and are listed rather than visualized in a larger context, which limit emphasis on semantic memory and working memory. In addition, to test visual organization and simple motor responses to visual stimuli, we selected the Weschler Intelligence Scale for Children (3rd version; WISC-III) Coding subtest for its emphasis on visuo-motor dexterity, processing speed, and visuo-spatial perceptual organization. The Coding subtest was preferred to the Symbol Search subtest (the other WISC-II test of processing speed), because the former is heavily influenced by grapho-motor demands and motor skills, while the latter carries less emphasis on visuo-motor dexterity and does not include information on motor responses to visual stimuli. Both the WJ-III Letter-Word Identification and WISC-II Coding subtests have been extensively used in developmental studies of cognition and show reliable, well-defined psychometric properties (Wechsler, 1991; Woodcock et al., 2001).

‘Secondary’, global attentional processes are involved in bringing an object into conscious awareness and planning goal-directed behavior, and are thought to be regulated, at least in part, by interactions between the amygdala and prefrontal cortex (Dolcos and McCarthy, 2006; Richards, 1998). To test whether primary dimensions of visual attention processes impact secondary, global attentional processes and executive control of complex goal-directed behaviors, we selected the Attention Problems subscale of the Child Behavior Checklist (CBCL) and Young Adult Self-Report (YASR). In addition, to rule out confounding effects of anxious-depressed symptoms (which may affect both primary and secondary attentional processes), we also tested for associations between DHEA, structural covariance and scores on the CBCL/YASR Anxious-Depressed subscale. The CBCL and YASR are age-appropriate instruments extensively used for assessing psychopathology and competence worldwide, and ask parents or young adults themselves to report on specific behaviors exhibited within the previous 6 months (Achenbach, 1991; Achenbach and Rescorla, 2001b). The YASR was derived from items on the CBCL and serves as a self-report extension of the CBCL for young adults (Achenbach, 1997). Both the CBCL and YASR are reliable measures with high stability over time, validated in multiple cultures (Achenbach and Rescorla, 2001a), with high internal consistency (Achenbach and Rescorla, 2001b).

2.5 Statistical Analyses

Statistical analyses were done using SurfStat (Matlab toolbox designed by Keith J. Worsley) and SPSS 21.0 (SPSS, Inc., Chicago, Illinois). Please see Table 2 for more details on statistical models used in this section.

2.5.1 Sample characteristics—We tested for differences in the complete sample used for brain analyses and the subsamples used for cognitive analyses using Chi-Squared goodness-of-fit and Student's T-tests, examining the distribution of scans, the number of males vs. females, age, total brain volume, pubertal stages, DHEA levels, collection times, handedness and amygdalar volumes. Differences across samples were considered significant at $p < 0.05$.

2.5.2 DHEA-Related Cortico-Amygdalar Networks—Mixed effects designs were used to model the relationship between DHEA and covariance of the amygdala with whole-brain, native-space CTh, taking into account the within- and between- individual variances in this longitudinal sample, and controlling for the effects of age, sex, total brain volume, scanner, handedness, time of salivary sampling. All continuous variables were centered using their respective means. A correction for multiple comparisons across the whole brain, using random field theory (RFT, $p < 0.05$), was applied to all analyses. In addition, p-values adjusted for False Discovery Rate (FDR) are listed in the Results and Legends section to account for analyses of the right, left and bilateral amygdala volumes (where $q < 0.05$ is considered significant; $q = \text{adjusted } p\text{-value} = p * n/r$; where 'p' is the unadjusted p-value; 'n' is the number of tests run; 'r' is the rank of the p-value). To examine associations between DHEA and structural covariance of the amygdala, we examined the significance of the term "DHEA*Amygdala", while controlling for all the aforementioned control variables (see example below, with the terms of interest underlined). We examined DHEA-related structural covariance of the right and left amygdala volumes, as well as the average volume of both amygdalae. To examine any distinct effects of DHEA above and beyond those related to estradiol, testosterone or season of collection, these variables were also included as control variables in additional DHEA-related models, and tested for interactions with DHEA, i.e. 'DHEA*testosterone*Amygdala'; 'DHEA*estradiol*Amygdala on whole-brain cortical thickness. Finally, to test for age effects on the relationship between DHEA and cortico-amygdalar networks, we tested for interactions with age, i.e. 'DHEA*Amygdala*Age' on whole-brain cortical thickness.

2.5.3 Cortico-Amygdalar Networks and Visual Attention—To examine associations between DHEA-related cortico-amygdalar networks and cognitive measures, we averaged the cortical thickness (CTh) of brain regions found to be significant in the previous section (see section 2.5.2) and examined the significance of the term "CTh*Amygdala" on cognitive measures, while controlling for all the aforementioned control variables (see example below, with the terms of interest underlined). More specifically, we tested for associations between: (1) amygdala-occipital covariance on visual awareness; (2) amygdala-parietal covariance on visuo-motor dexterity; and (3) amygdala-prefrontal covariance on attention, in accordance with results from section 2.5.2 and based on previous evidence of the relationship between these brain regions and visual attention (Graydon et al., 2005; Pessoa, 2008; Phelps and

LeDoux, 2005). To rule out a confounding effect of anxious-depressed symptoms (which may affect primary and secondary attentional processes), we also tested for associations between each of the DHEA-related cortico-amygdalar networks and anxious-depressed symptoms. Of note, because only the brain relationships found to be significant in section 2.5.2 (amygdala-cortical pairs) were examined in section 2.5.3, there was no need to apply FDR or RFT adjustments to control for multiple comparisons.

2.5.4 Mediation Effects of Cortico-Amygdalar Networks—We formally tested whether cortico-amygdalar covariance could mediate the relationship between DHEA and tests of visual attention. To examine the relationship between DHEA and cortico-amygdalar networks, we extracted the coefficients and p-values of the significant interaction term ‘DHEA*Amygdala’ for peak vertices (i.e. vertices with the highest coefficient; see section 2.5.2 for more details). Of note, the full range of coefficients for vertices within the area of significance is also listed (see Legend of Figure 4); numbers did not significantly differ from those listed for peak vertices. To examine the relationship between DHEA-related cortico-amygdalar networks and visual attention, we extracted the coefficients and p-values of the significant interaction term ‘CTh*Amygdala’ (see section 2.5.3 for more details). It is important to emphasize that no additional analyses were run for those two sections of the mediation analysis other than those already described above (see sections 2.5.2 and 2.5.3).

Additional analyses were run to test the relationship between DHEA and measures of visual attention, using mixed effect models in SPSS, and controlling for age and sex. Finally, coefficients and p-values were extracted from existing analyses, and entered in the Sobel-Goodman test calculator to formally test mediation effects (<http://quantpsy.org/sobel/sobel.htm>). This more traditional approach to test mediation and moderation effects, using Baron-Kenney’s criteria and augmented by a formal Sobel’s test, was preferred by our group to more recent methods that include bootstrapping. This is because of the complexity of our longitudinal data (multiple scans per subjects, different number of scans per subject). The traditional method treats each relationship (between predictor and moderator, and then between moderator and outcome) separately, allowing us to model the longitudinal component of the data. Finally, please note that the same set of control variables (including age), as listed in sections 2.5.2 and 2.5.3 was used for the mediation analyses.

2.5.5 Relationship between Primary and Secondary Measures of Visual Attention—We examined the relationship between primary measures of visual awareness (WJ-III Letter Word Identification), visuo-motor dexterity (WISC-III Coding scores) and global attentional deficits, as measured by the CBCL Attention Problems subscale. Principal component analysis was used to extract a common factor representing primary dimensions of visual processing from the combination of visual awareness and visuo-motor dexterity scores. Mixed effects designs were subsequently used to model the relationship between the primary visual component and the CBCL Attention Problems subscale, taking into account the within- and between- individual variances in this longitudinal sample, and controlling for the effects of age and sex.

2.5.6 Pubertal Effects on Cortico-Amygdalar Networks and Visual Attention—It is important to distinguish the effects of pubertal status from those of DHEA on cortico-

amygdalar networks and on the relationship between structural covariance and visual attention. However, there is a complex relationship between DHEA, age and pubertal stage, such that, at ages lower than 13 years old, there is a positive relationship between DHEA and pubertal stage (r-squared, linear model: 0.124); however, at higher age groups, the association between DHEA and pubertal stage is no longer significant (r-squared, linear model: 0.002). Because of this relationship, a problem of collinearity arises in the younger age group if both variables are included in the same model. Therefore, pubertal effects on cortico-amygdalar covariance and cognitive measures were examined in separate models (similar to those used for DHEA, see section 2.5.2).

3. RESULTS

3.1 Sample Characteristics

Table 1 details sample characteristics, including number of longitudinal scans and covariates of interest. The sample used for DHEA-related analyses included 216 participants (F= 190, M=134), and 324 scans. Participants were aged between 6 and 22 years old, with a mean age of 13.5 (SD = 3.1 years). There were also no significant differences in the total number of scans, distribution of scans across individuals (1, 2, or 3 MRI scans), DHEA levels, collection times, number of boys and girls, total brain volumes, or amygdala volumes across samples. There were significant differences in mean age and pubertal status across samples between the group of subjects used for DHEA-related analyses and three of the samples used for cognitive analyses (see Table 1 for more details). In order to account for these differences, age was included as a covariate in all analyses, and pubertal effects on cortico-amygdalar covariance and visual attention were assessed in separate models (see section 2.5 for more details).

3.2 DHEA-Related Cortico-Amygdalar Networks

As shown in Figure 1a, whole-brain analyses, controlling for the effects of age, sex, total brain volume, scanner, handedness and collection time of salivary samples, revealed that DHEA is associated with the structural covariance between both the left and right amygdala and CTh of the left occipital pole (linear models, Brodmann 17, cluster-level $p=0.003$, 312 voxels, peak vertex id 33120 [$x=-7.09$, $y=-102.1$, $z=5.2$], $p=7*10^{-4}$; adjusted p-value (FDR)= $1*10^{-3}$). As shown in Figure 1b, lower DHEA levels (<2.22 pg/mL) were associated with a positive covariance between the amygdala and CTh of this occipital region, and higher DHEA levels (>2.22 pg/mL) were associated with negative amygdala-occipital covariance.

As shown in Figure 2a, DHEA was also found to be associated with the structural covariance between the right amygdala and CTh of the right somatosensory cortex (linear models, Brodmann 1, 2, 3 and 5, cluster-level $p=0.003$, 144 voxels, peak vertex id 71795 [$x=5.6$, $y=-37.4$, $z=55.1$], $p=1*10^{-4}$; adjusted p-value (FDR)= $3*10^{-4}$). As shown in Figure 2b, lower DHEA levels (<2.22 pg/mL) were associated with a positive covariance between the amygdala and CTh of this parietal region, and higher DHEA levels (>2.22 pg/mL) were associated with negative amygdala-parietal covariance.

Lastly, as shown in Figure 3a, DHEA was found to be associated with the structural covariance between the right amygdala and CTh of the right subgenual anterior cingulate cortex (linear models, Brodmann 25, cluster-level $p=0.017$, 47 voxels, peak vertex id 56613 [$x=3.6$, $y=17.0$, $z=-8.5$], $p=1*10^{-3}$, adjusted p-value (FDR)= $1*10^{-3}$). As shown in Figure 3b, lower DHEA levels (<2.22 pg/mL) were associated with a positive covariance between the amygdala and CTh of this frontal region, and higher DHEA levels (>2.22 pg/mL) were associated with negative amygdala-prefrontal covariance.

No other brain region met the threshold for significance (RFT, $p<0.05$). Adding estradiol, testosterone, and season of sampling as control variables did not result in any differences in the above findings. Finally, there were no significant interactions between DHEA and age, testosterone, or estradiol on cortico-amygdalar networks.

3.3 Cortico-Amygdalar Networks and Visual Attention

As shown in Figure 1c, analyses controlling for the effects of age, sex, total brain volume, scanner and handedness, revealed that visual awareness, as measured by scores on the WJ-III: Letter Word Identification test, was related to structural covariance between both left and right amygdalae and the left occipital pole (linear models, Brodmann 17, cluster-level $p=3*10^{-3}$). Lower cognitive scores (<60) were associated with a positive covariance between the amygdala and CTh of this occipital region (similar to the covariance seen with lower DHEA levels), and higher cognitive scores (>60) were associated with negative amygdala-occipital covariance (similar to the covariance seen with higher DHEA levels).

As shown in Figure 2c, visuo-motor dexterity, as measured by scores on the WISC-III: Coding test, was related to structural covariance between the right amygdala and CTh of the right somatosensory cortex (linear models, Brodmann 5, 1, 2, and 3, $p=1*10^{-3}$). Lower cognitive scores (<10) were associated with a positive covariance between the amygdala and CTh of this parietal region (similar to the covariance seen with lower DHEA levels), and higher cognitive scores (>10) were associated with negative amygdala-parietal covariance (similar to the covariance seen with higher DHEA levels).

As shown in Figure 3c, attentional deficit, as measured by scores on the CBCL Attention Problems scale, was related to structural covariance between the right amygdala and CTh of the right subgenual anterior cingulate cortex (linear models, Brodmann 25, $p=4*10^{-2}$). Higher scores on the Attention Problems scale (scores >5) were associated with a positive covariance between the amygdala and this prefrontal region (similar to the covariance seen with lower DHEA levels). Lower scores on the Attention Problems scale (scores <5) were associated with a negative amygdala-prefrontal covariance (similar to the covariance seen with higher DHEA levels).

Of note, no significant relationship emerged between any of the cortico-amygdalar networks and anxious-depressed symptoms.

3.4 Mediation Effects of Cortico-Amygdalar Networks

As shown in Figure 4, the relationships between DHEA and cortico-amygdalar covariance and the relationships between cortico-amygdalar covariance and measures of visual attention

were more significant and of greater magnitude than the relationship between DHEA and measures of visual attention. Though the relationship between DHEA and visual attention measures was not found to be significant, this does not rule out a mediation effect of cortico-amygdalar networks on the relationship between DHEA and visual attention. Indeed, such mediating effects (i.e. between DHEA and cortico-amygdalar networks on one hand, and cortico-amygdalar networks and visual attention on the other) can exist independently from the relationship between DHEA and visual attention—as previously demonstrated mathematically (Hayes, 2009). Indeed, formal tests (Sobel-Goodman) of these mediating effects were shown to be significant for primary dimensions of visual attention, with a notable trend for secondary, global attentional deficits (see Figure 4). Taken together, these results suggest that cortico-amygdalar networks may mediate the relationship between DHEA and different components of visual attention.

3.5 Relationship between Primary and Secondary Measures of Visual Attention

Scores on the primary measures of visual awareness (WJ-III Letter-Word Identification subtest) and visuo-motor dexterity (WISC-III Coding subtest) loaded significantly to a common component (factor loadings=0.7 for both measures, explaining 50.6% of the variance between the two tests). Typically, factor loadings of more than 0.4 and explaining more than 16% of the variance are considered significant (Stevens, 2002). Scores on the secondary, global measure of attention (CBCL Attention Problems) were inversely related to the common component extracted from visual awareness and visuo-motor dexterity (beta-coefficient for the common=-0.4, SE=0.1, F=208.0, p=0.004).

3.6 Pubertal Effects on Cortico-Amygdalar Networks and Visual Attention

Pubertal stage did not have any significant effects on structural covariance of the amygdala with cortical thickness across the whole brain. Similarly, pubertal stage did not have any significant effects on the relationship between cortico-amygdalar covariance and cognitive measures.

4. DISCUSSION

This report provides evidence that DHEA may regulate visual attention through structural plasticity of cortico-amygdalar networks. We found a significant inverse relationship between DHEA and structural covariance of the amygdala with occipital, parietal and prefrontal regions. These effects of DHEA were above and beyond those of age and sex, as well as distinct from those of pubertal stage, estradiol and testosterone, supporting the notion that DHEA carries direct and specific actions on limbic and cortical regions during human development.

The negative covariance in cortico-amygdalar networks seen at higher DHEA levels was associated with higher scores on measures of visual attention, as well as decreased attentional impairment, supporting the notion that stimulant-like effects of DHEA may be independent of developmental stage (Strous et al., 2001). Prior studies have linked these pro-cognition effects of DHEA to a functional decrease in amygdala-induced salience for emotional stimuli (Sripada et al., 2013; Wolf et al., 1998). Thus, taken together with these

prior observations, results from this study suggest that structural cortico-amygdalar plasticity may underlie, and perhaps precede, changes in amygdala function related to DHEA.

Although the amygdala has been frequently labeled as a ‘fear center’, accumulating evidence points to its important role in increasing the salience of affective stimuli, to the detriment of non-affective, or more purely ‘cognitive’ processes (Pessoa, 2008). In addition, amygdalar plasticity in response to external stimuli may also alter plasticity in sensory cortical regions in a modality-specific manner (Phelps and LeDoux, 2005). The basolateral division of the amygdala is made of cells resembling cortical neurons, and connects directly to fronto-temporal networks, making these nuclei the most likely target for DHEA-related cortico-amygdalar effects (Sah et al., 2003). This concept is further supported by findings of negative functional coupling between the basolateral amygdala and the same regions found here to show DHEA-related negative structural covariance with the amygdala (medial prefrontal, parietal and occipital cortices) (Gabard-Durnam et al., 2014). This phenomenon may be particularly far-reaching during childhood and adolescence, when early emerging ‘bottom-up’ systems mature faster than ‘top-down’ cognitive control regions (Casey and Jones, 2010).

DHEA levels reach their peak during early brain development and may therefore carry great impact on amygdala-dependent plasticity of sensory cortical regions, perhaps through a strengthening of neuronal and glial connections between cortical and limbic regions. Several mechanisms could account for DHEA’s effects on the amygdala, including, but not limited to, its anti-glucocorticoid and antioxidant effects (Campbell, 2011; Yorek, 2002), its GABA_A agonist effects, and its upregulation of brain-derived neurotrophic factor (BDNF) concentration and serotonin (5-HT_{2A}) receptor expression (Cyr et al., 2000; Maninger et al., 2009; Naert et al., 2007; Park-Chung et al., 1999). In particular, anti-glucocorticoid actions of DHEA may act both to decrease excessive CNS glucose utilization and neurotoxic effects of glucocorticoids in ‘bottom-up’ systems during development, again enhancing the potential for long-range, ‘top-down’ connections to develop in the future (Campbell, 2011). Similarly, GABA-ergic actions of DHEA may be involved in downregulating amygdalar activity and ‘bottom-up’ activation of cortico-amygdalar networks, perhaps in order to preserve the potential for ‘top-down’ connections to form later on (Campbell, 2011; Park-Chung et al., 1999). Taken together, these mechanisms support the biological plausibility and evolutionary advantage of a covariance between DHEA, amygdalar volume, and cortical brain systems involved in sensory and attentional processes.

More specifically, higher DHEA levels were associated with a negative covariance between amygdala-occipital regions; in turn, negative covariance between these regions was associated with higher visual awareness scores. Findings that DHEA-related amygdala-occipital covariance is associated with reading and visual decoding abilities suggest a potential role for DHEA in the filtering of salient visual stimuli and visual awareness. The visual cortex and the amygdala both show an increase in activity in response to affective stimuli, such as emotional faces (Kim et al., 2011), suggesting robust connections between these regions. In fact, efferent projections from the amygdala to the primary visual cortex far outnumber connections coursing in the opposite direction (Amaral et al., 1992; Freese and Amaral, 2005), supporting the notion that amygdalar activity modulates visual processing

and prioritization of attention, selecting salient stimuli for privileged access to visual awareness, even prior to the conscious detection of visual stimuli. DHEA-related alterations in amygdalar structure and covariance with cortical visual networks may therefore play a role in downregulating amygdala-dependent neural bias in visual processing related to affect modulation.

A similar relationship was also present for amygdala-parietal covariance (i.e. higher DHEA→negative amygdala-parietal covariance→higher visuo-motor processing scores). Findings that DHEA-related amygdala-parietal covariance is associated with visuo-motor processing speed suggest a potential role for DHEA in the regulation of motor responses to visual stimuli. The amygdala has been shown to modulate the response of the inferior parietal cortex to pre-conscious processing of visual information (Troiani and Schultz, 2013), suggesting that ‘bottom-up’, amygdala-dependent regulation of parietal regions may significantly impact visual processing. Consistent with the role of the parietal cortex in early visuo-spatial attention (Goodale and Milner, 1992; Mishkin and Ungerleider, 1982), these results further suggest that DHEA may be involved in modulating amygdala-dependent plasticity of the parietal cortex, perhaps through a downregulation of sensorimotor bias related to affect modulation.

Finally, we found significant associations between DHEA, amygdala-prefrontal covariance and attention problems (i.e. higher DHEA→negative amygdala-prefrontal covariance→improved attention). In contrast to adult samples, ‘bottom-up’ functional connectivity from the amygdala to the subgenual anterior cingulate cortex was found to be much more significant than ‘top-down’ functional connectivity in both healthy and depressed adolescents (Musgrove et al., 2015), supporting the notion that amygdala-dependent regulation of cortical processes may predominate in this age group. Interestingly, several studies have documented the significant changes in amygdala-prefrontal circuitry from childhood to adulthood as a function of age (Gabard-Durnam et al., 2014; Gee et al., 2013; Perlman and Pelphrey, 2011; Swartz et al., 2014). Some, though not all studies, have documented a shift from positive to negative amygdala-prefrontal coupling with increasing age (Gee et al., 2013), consistent with the shift from positive to negative covariance observed here with higher DHEA levels. This may suggest that at least some of the effects previously ascribed to age can be attributed to varying DHEA levels during development. Still, the subgenual ACC has been broadly implicated in global attentional processes and emotional regulation, and changes in its structural or functional connectivity are likely to be, for the better part, a result of age-related whole-brain maturation rather than specific hormonal effects (Etkin et al., 2011). This may explain the lower significance of the relationship between DHEA, amygdala-prefrontal covariance and attention problems in this sample, compared to brain-cognition associations involving amygdala-occipital or amygdala-parietal networks. Nevertheless, based on the current results, one could speculate that DHEA-related alterations in amygdalar-prefrontal networks may contribute to decrease the bias in attentional processing due to affect modulation.

Interestingly, the relationship between primary measures of visual attention and secondary attentional deficits at the behavioral level provides further support to the hypothesis that early stage, primary disruption at the perceptual level can generalize ‘upward’ to erode

global cognitive processes (Butler et al., 2005). These findings are consistent with reports of early-stage visual processing deficits and cortical amplification processes leading to an impact on sustained attention, problem-solving, independent living and global functioning in patients with schizophrenia (Prouteau et al., 2004). Similarly, another study reported a significant relationship between deficits in primary visual perception and global attention in the same patient group (Lee and Cheung, 2005). A group of investigators found improvement of visuo-motor skills and sustained attention, but no change in any other clinical symptom, after DHEA augmentation of antipsychotic treatment (Ritsner et al., 2006). Here we found more significant relationships between DHEA-related cortico-amygdalar networks and primary measures of visual attention, compared to the relationships between DHEA-related structural covariance and behavioral measures such as attention and anxious-depressed symptoms. Taken together with the results from previous investigations, one could speculate that DHEA preferentially impacts primary, perceptual-level cognitive processes, a phenomenon that may then generalize to affect general attentional processes.

4.1 Limitations

The scans were done on 1.5T scanners, which have lower resolution compared to newer 3T models. This could have increased the risk of systematic errors when data is automatically processed. Still, all quality-controlled structural MR images were processed using the highest standards (see section 2.2). Another limitation concerns the lack of functional MRI data available to directly test the relationship between structural covariance and functional connectivity in this sample. Nonetheless, the structural brain networks identified here partially overlap with regions whose functional connectivity was previously linked to DHEA administration or amygdala resting-state networks (Sripada et al., 2013; Sripada et al., 2014). Another common concern in hormone-brain association studies is the presence of intra-individual hormonal variation due to known, or unknown, causes. Reassuringly, androgen levels have been shown to remain highly correlated for several days, weeks and possibly even an entire year, and to reliably correlate with stable measures of personality (Dabbs Jr, 1990; Granger et al., 2004; Sellers et al., 2007). Still, to limit any systematic bias related to intra-individual hormonal variation, we have controlled for sex, diurnal and seasonal variation, with no significant changes in the results. Another limitations is the lack of umbilical cord or amniotic measurements were available in this study and we therefore cannot control for DHEA levels *in utero*, a period during which significant DHEA-related changes in brain structure may occur. However, more recent observations have identified continuing and distinct organizational effects of DHEA throughout childhood and adolescence, supporting the functional importance of postnatal brain changes related to DHEA, above and beyond those restricted to the prenatal period (Nguyen et al., 2013b). Finally, behavioral measures of secondary, global attention processes relied on self- and parental reports rather than direct behavioral measurements. Still, the measure used (CBCL) has high internal consistency and stability over time (Achenbach and Rescorla, 2001b), has been validated in multiple cultures (Achenbach and Rescorla, 2001a), and has been shown to demonstrate adequate diagnostic accuracy in the screening of attention problems (Aebi et al., 2010).

4.2 Conclusions

DHEA may play a unique evolutionary role in the brain development of humans and the great apes. Here we found DHEA to be involved in regulating human-specific visual attention systems by reshaping cortico-amygdalar networks from middle childhood to young adulthood. Effects of DHEA were above and beyond those of sex and age, as well as distinct from those of pubertal stage, estradiol and testosterone, supporting the notion that DHEA may uniquely regulate ‘bottom-up’ amygdala-dependent cortical plasticity and visual attention systems in humans.

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Appendix A

The Brain Development Cooperative Group is a multi-site research group formed from personnel from several pediatric study centers.

Data was collected from 6 sites across the United States:

1. Children's Hospital Medical Center of Cincinnati, Cincinnati, OH, USA, 45229:
Principal Investigator William S. Ball, M.D., Investigators Anna Weber Byars, Ph.D., Mark Schapiro, M.D., Wendy Bommer, R.N., April Carr, B.S., April German, B. A., and Scott Dunn, R.T.;
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3. University of Texas Health Science Center at Houston, Houston, TX, USA, 77030: :
Principal Investigators Michael E. Brandt, Ph.D., Jack M. Fletcher, Ph.D., and Larry A. Kramer, M.D., Investigators Grace Yang, M.Ed., Cara McCormack, B.S., Kathleen M. Hebert, M.A., and Hilda Volero, M.D.;
4. Washington University in St. Louis, St. Louis, MO, USA, 63110:
Principal Investigators Kelly Botteron, M.D. and Robert C. McKinstry, M.D., Ph.D., Investigators William Warren, Tomoyuki Nishino, M.S., C. Robert Almlie, Ph.D., Richard Todd, Ph.D., M.D., and John Constantino, M.D.;
5. University of California Los Angeles, Los Angeles, CA, USA, 90024:
Principal Investigator James T. McCracken, M.D., Investigators Jennifer Levitt, M.D., Jeffrey Alger, Ph.D., Joseph O'Neil, Ph.D., Arthur Toga, Ph.D., Robert

Asarnow, Ph.D., David Fadale, B.A., Laura Heinichen, B.A., and Cedric Ireland B.A.;

6. Children's Hospital of Philadelphia, Philadelphia, PA, USA, 19104:
Principal Investigators Dah-Jyuu Wang, Ph.D. and Edward Moss, Ph.D.,
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Highlights

- Adrenarche is a developmental event unique to humans and the great apes
- DHEA reaches its highest circulating levels during adrenarche
- DHEA regulates structural plasticity of cortico-amygdalar networks during development
- DHEA-related cortico-amygdalar networks regulate visual attention during development
- Effects of DHEA are distinct from those of sex, age, puberty, estradiol, testosterone

Figure 1. DHEA-Related Amygdala-Occipital Covariance and Relationship with Visual Awareness

This figure shows cortico-amygdalar covariance related to DHEA levels and to visual awareness (Figure 1a, Graph 1b, Graph 1c). Higher DHEA levels are associated with a negative covariance between amygdalar volume and cortical thickness of the left occipital pole (Graph 1b). In turn, negative covariance between these regions is associated with higher scores on a test of visual awareness (Graph 1c).

Figure 1a shows the region of the left occipital cortex (primary visual cortex, Brodmann 17) where the relationship between cortical thickness and amygdalar volume (average of the left and right amygdalar volumes) significantly varies according to DHEA levels (surviving correction for multiple comparisons across the whole brain using random-field-theory $p < 0.05$).

Graph 1b displays the relationship between amygdalar volume (average of the left and right amygdalae, X axis) and cortical thickness of the primary visual cortex (Y axis) for subjects

with lower DHEA (<2.22 pg/mL; black lines) and higher DHEA (>2.22 pg/mL; red lines) levels.

Graph 1c displays the relationship between amygdalar volume (average of the left and right amygdalae, X axis) and cortical thickness of the primary visual cortex (Y axis) for subjects with lower visual awareness (<60; black lines) and higher visual awareness (>60; red lines) scores.

Note that standardized residuals (accounting for the effects of age, sex, handedness, scanner, and total brain volume in all analyses, as well as collection time for DHEA-related analyses) were used for cortical thickness values on the Y axes of Graphs 1b and 1c. Occipital CTh stands for cortical thickness of the occipital region displayed in Figure 1a.

DHEA and cognitive scores were split into high and low groups for the purposes of visualization, based on the values at which cortico-amygdalar covariance shifted from positive to negative. Both variables were included as continuous variables in all analyses.

Figure 2. DHEA-Related Amygdala-Parietal Covariance and Relationship with Visuo-Motor Dexterity

This figure shows cortico-amygdalar covariance related to DHEA levels and to visuo-motor dexterity (Figure 2a, Graph 2b, Graph 2c). Higher DHEA levels are associated with a negative covariance between the volume of the right amygdala and cortical thickness of the right parietal cortex (Graph 2b). In turn, negative covariance between these regions is associated with higher scores on a test of visuo-motor dexterity (Graph 2c).

Figure 2a shows the region of the right parietal cortex (primary and association somatosensory cortex, Brodmann 1, 2, 3 and 5) where the relationship between cortical thickness and volume of the right amygdala significantly varies according to DHEA levels (surviving correction for multiple comparisons across the whole brain using random-field-theory $p < 0.05$).

Graph 2b displays the relationship between volume of the right amygdala (X axis) and cortical thickness of the primary and association somatosensory cortex (Y axis) for subjects with lower DHEA (<2.22 pg/mL; black lines) and higher DHEA (>2.22 pg/mL; red lines) levels.

Graph 2c displays the relationship between volume of the right amygdala (X axis) and cortical thickness of the primary and association somatosensory cortex (Y axis) for subjects with lower visuo-motor dexterity (<10; black lines) and higher visuo-motor dexterity (>10; red lines) scores.

Note that standardized residuals (accounting for the effects of age, sex, handedness, scanner, and total brain volume in all analyses, as well as collection time for DHEA-related analyses) were used for cortical thickness values on the Y axes of Graphs 2b and 2c. Parietal CTh stands for cortical thickness of the parietal region displayed in Figure 2a.

DHEA and cognitive scores were split into high and low groups for the purposes of visualization, based on the values at which cortico-amygdalar covariance shifted from positive to negative. Both variables were included as continuous variables in all analyses.

Figure 3. DHEA-Related Amygdala-Prefrontal Covariance and Relationship with Attention Problems

This figure shows cortico-amygdalar covariance related to DHEA levels and to scores of visual awareness (Figure 3a, Graph 3b, Graph 3c). Higher DHEA levels are associated with a negative covariance between the volume of the right amygdala volume and cortical thickness of the right subgenual anterior cingulate cortex (Graph 3b). In turn, negative covariance between these regions is associated with lower scores on a test of attentional impairment (Graph 3c).

Figure 3a shows the region of the right prefrontal cortex (subgenual anterior cingulate cortex, Brodmann 25) where the relationship between cortical thickness and volume of the right amygdala significantly varies according to DHEA levels (surviving correction for multiple comparisons across the whole brain using random-field-theory $p < 0.05$).

Graph 3b displays the relationship between volume of the right amygdala (X axis) and cortical thickness of the subgenual anterior cingulate cortex (Y axis) for subjects with lower DHEA (<2.22 pg/mL; black lines) and higher DHEA (>2.22 pg/mL; red lines) levels. Graph 3c displays the relationship between volume of the right amygdala (X axis) and cortical thickness of the subgenual anterior cingulate cortex (Y axis) for subjects with lower attention problems (<5; black lines) and higher attention problems (>5; black lines). Note that standardized residuals (accounting for the effects of age, sex, handedness, scanner, and total brain volume in all analyses, as well as collection time for DHEA-related analyses) were used for cortical thickness values on the Y axes of Graphs 3b and 3c. ACC CTh stands for cortical thickness of the anterior cingulate region displayed in Figure 3a. DHEA and cognitive scores were split into high and low groups for the purposes of visualization, based on the values at which cortico-amygdalar covariance shifted from positive to negative. Both variables were included as continuous variables in all analyses.

Sample characteristics

TABLE 1

	DHEA	WJ-III: Letter word	WISC-III: Coding	CBCL: Attention Problems	CBCL: Anxious-Depressed
Measures	Total Sample n=216	Total Sample n=210	Total Sample n=192	Total Sample n=207	Total sample n=207
1 MRI scan	324 scans n=129 129 scans	305 scans $\chi^2=1.1$ p=0.6	261 scans $\chi^2=6.1$ p=0.05	290 scans $\chi^2=2.7$ p=0.3	290 scans $\chi^2=2.7$ p=0.2
2 MRI scan	n=66 132 scans	n=135 135 scans	n=135 135 scans	n=139 139 scans	n=139 139 scans
3 MRI scan	n=21 63 scans	n=55 110 scans	n=45 90 scans	n=53 106 scans	n=53 106 scans
DHEA (pg/mL)	159.2 SD 152.6	n=20 60 scans 158.5 SD 154.8 F=0.2 p=0.6	n=12 36 scans 135.9 SD 136.0 F=0.1 p=0.8	n=15 45 scans 143.4 SD 137.8 F=3.3 p=0.07	n=15 45 scans 143.4 SD 137.8 F=3.2 p=0.08
Collection time (min) after midnight)	697.8 SD 127.3	697.1 SD 130.1 F=0.03 p=0.9	695.4 SD 125.0 F=0.6 p=0.5	698.3 SD 125.2 F=1.4 p=0.2	698.3 SD 125.2 F=1.1 p=0.3
Age (years)	13.5 Range 6.1–22.3 SD 3.6	13.5 Range 6.1–22.3 SD 3.6 F=1.1 p=0.3	12.3 Range 6.1–16.9 SD 2.8 F=148.2 p<0.0001 *	12.8 Range 6.1–19.7 SD 3.1 F=82.4 p<0.0001 *	12.8 Range 6.1–19.7 SD 3.1 F=78.5 p<0.0001 *
Gender F=female M=male	F=190 M=134	F=178 M=127 $\chi^2=0.04$ p=0.8	F=154 M=107 $\chi^2<0.001$ p=1.0	F=172 M=118 $\chi^2<0.001$ p=1.0	F=172 M=118 $\chi^2<0.001$ p=1.0
Pubertal	2.7 Range 1–5 SD 1.5	2.6 Range 1–5 SD 1.5 F=2.5 p=0.1	2.6 Range 1–5 SD 1.5 F=8.0 p=0.005 *	2.6 Range 1–5 SD 1.5 F=19.7 p<0.0001 *	2.6 Range 1–5 SD 1.5 F=20.7 p<0.0001 *
Handedness	R=299 L=25	R=281 L=24 $\chi^2=0.0$ p=1.0	R=240 L=21 $\chi^2=0.0$ p=1.0	R=266 L=24 $\chi^2=0.0$ p=1.0	R=266 L=24 $\chi^2=0.0$ p=1.0

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Measures	DHEA	WJ-III: Letter word		WISC-III: Coding		CBCL: Attention Problems		CBCL: Anxious-Depressed	
		Total Sample	n=210	Total Sample	n=192	Total Sample	n=207	Total sample	n=207
Total brain volume (cm ³)	1274.2 SD 129.1	1275.9 SD 128.6 F=2.2 p=0.1		1280.8 SD 128.8 F=0.4 p=0.5		1276.4 SD 1306.7 F=0.3 p=0.6		1276.4 SD 1306.7 F=1.0 p=0.3	
Left amygdala (mm ³)	1074.8 SD 131.9	1077.3 SD 132.1 F=1.4 p=0.2		1077.0 SD 131.2 F=2.9 p=0.1		1073.7 SD 133.0 F=2.2 p=0.1		1073.7 SD 133.0 F=2.8 p=0.1	
Right amygdala (mm ³)	1095.0 SD 123.9	1095.5 SD 124.1 F=1.0 p=0.3		1092.0 SD 124.0 F=0.003 p=1.0		1092.6 SD 124.0 F=1.3 p=0.3		1092.6 SD 124.0 F=1.9 p=0.2	
Cognitive Tests		62.3 Range 18–76 SD 9.9		10.4 Range 1–18 SD 3.3		1.6 Range 0–10 SD 2.0		1.7 Range 0–11 SD 2.0	

Note that all values have been rounded to the nearest decimal

* Significant comparisons p<0.05

Table 2

Description of statistical models

Methods section	Statistical model
2.5.2 DHEA & Cortico-Amygdalar Networks	1 Whole-brain CTh = 1 + <u>DHEA</u> * <u>Amygdala</u> + DHEA + Amygdala + Collection Time + Age + Sex + Scanner + Handedness + Total Brain Volume + random (Subj) + I
	2 Interactions between DHEA and estradiol, testosterone, season of collection and age
2.5.3 Cortico-Amygdalar Networks & Visual Attention	1 Cognitive Scores = 1 + <u>CTh</u> * <u>Amygdala</u> + CTh + Amygdala + Age + Sex + Scanner + Handedness + Total Brain Volume + random (Subj) + I
2.5.4 Mediation	1 Coefficients and p-values extracted from section 2.5.2
	2 Coefficients and p-values extracted from section 2.5.3
	3 Cognitive scores = 1 + <u>DHEA</u> + Age + Sex + random (Subj) + I
	4 Coefficients and p-values from (1), (2) and (3) were extracted from existing analyses, and entered in the Sobel-Goodman test calculator to formally test mediation effects of scans per subject (see 'Methods')
2.5.5 Primary & Secondary Attention	1 Principal component analysis was used to extract a common factor representing primary dimensions of visual processing from the combination of visual awareness and visuo-motor dexterity scores
	2 CBCL Attention Problems = 1 + <u>Visual_attention</u> + Age + Sex + random (Subj) + I
2.5.6 Puberty & Cortico-Amygdalar Networks	Whole-brain CTh= 1 + <u>Pubertal Stage</u> * <u>Amygdala</u> + Pubertal Stage + Amygdala + Age + Sex + Scanner + Handedness + Total Brain Volume + random (Subj) + I*
2.5.7 Puberty, Cortico-Amygdalar Networks & Visual Attention	Cognitive Scores= 1 + <u>Pubertal Stage</u> * <u>Amygdala</u> * <u>CTh</u> + Pubertal Stage* <u>Amygdala</u> + Pubertal Stage*CTh + Amygdala*CTh + Pubertal Stage + Amygdala + CTh + Age + Sex + Scanner + Handedness + Total Brain Volume + random (Subj) + I*

'Subj' refers to a specific subject; and 'I' to the identity matrix of the mixed effects model

'CTh' as referred to in section 2.5.3 and 2.5.7 refers to average cortical thickness of brain areas found to be significant in section 2.5.2

'Visual Attention' refers to the common component of visual awareness and visuo-motor dexterity scores; 'Subj' refers to a specific subject; and 'I' to the identity matrix of the mixed effects model