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Relationships between adrenarcheal hormones, hippocampal volumes and depressive symptoms in children

Rachel Ellis[a,1], Anna Fernandes[a,b,1,2], Julian G. Simmons[a,b], Lisa Mundy[d,e], George Patton[d,e], Nicholas B. Allen[b,c], Sarah Whittle[a,b,*]

[a] Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne and Melbourne Health, Parkville, VIC, Australia
[b] Melbourne School of Psychological Sciences, The University of Melbourne, Parkville, VIC, Australia
[c] Department of Psychology, University of Oregon, Eugene, OR, USA
[d] Department of Paediatrics, The University of Melbourne, Parkville, VIC, Australia
[e] Centre for Adolescent Health, Murdoch Children's Research Institute, Parkville, VIC, Australia

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ABSTRACT

Early timing of puberty (i.e., advanced pubertal maturation relative to same-age peers) has been associated with depressive symptoms during adolescence. To date, research on this relationship has focused on gonadarche, the second phase of puberty, while less is known about the first phase of puberty, adrenarche. Increasing evidence suggests that androgens that rise during adrenarche, most notably dehydroepiandrosterone (DHEA) and testosterone, may be involved both in the development of the hippocampus, and risk for depression. The current study investigated whether hippocampal volumes mediated the relationship between adrenarcheal timing (based on relative levels of adrenarcheal hormones) and depressive symptoms in children. Data were collected from a cross-sectional sample of 88 children (46 female) selected to have relatively increased variance in these androgens. Participants completed brain MRI structural scans, provided saliva samples for hormones, and completed the Children's Depression Inventory (CDI). Contrary to predictions, larger right hippocampi significantly partially mediated the positive relationship between early timing of testosterone exposure (i.e., relatively high levels of testosterone for one's age) and depressive symptoms in girls. No other evidence of significant mediation effects was obtained, however DHEA and testosterone exposure showed unique effects on hippocampal volumes in males and females, and larger hippocampal volumes predicted higher depressive symptoms in the entire sample. These results suggest that adrenarcheal timing may be related to hippocampal development and depressive symptoms, extending current knowledge of pubertal risk processes.

1. Introduction

Puberty is a critical maturational period when increases in adrenarcheal and gonadarcheal hormones occurs alongside considerable physical and neural development, and associated changes in cognition, affect, and behavior (Patton and Viner, 2007). Although many individuals navigate the pubertal transition without significant difficulty, there is a striking rise in mental health difficulties during this time. In particular, the prevalence of depressive symptoms and disorders increases significantly from early adolescence, and elevated depressive symptoms have long term implications for psychosocial dysfunction (Lewinsohn et al., 2000).

The pubertal transition is a complex process with a long genesis, comprising at least three different endocrine processes: adrenarche, gonadarche, and activation of the growth axis. The outward signs of gonadarche are often taken to represent puberty per se, and thus gonadarche has been the predominant focus of research exploring links between puberty and adverse adolescent outcomes (Blakemore et al., 2010). Adrenarche typically begins between ages 6 and 9 years, prior to the onset of gonadarche (Grumbach and Styne, 2011). Adrenarche is characterized by reactivation of the hypothalamic-pituitary-adrenal (HPA) axis, reflected in the rise of the adrenal androgens dehydroepiandrosterone (DHEA), its sulfated ester (DHEAS), and testosterone via conversion of DHEA in the adrenals and peripheral tissue.

* Corresponding author at: Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne, Level 3, 161 Barry Street, Carlton, VIC, 3053, Australia.
E-mail address: swhittle@unimelb.edu.au (S. Whittle).
[1] Joint first authorship.
[2] Present address: Ballarat Health Services, Ballarat, VIC, Australia.

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Differences in the timing of pubertal maturation have been repeatedly associated with depressive symptoms. Specifically, early gonadarche (i.e., early signs of gonadarcheal maturation relative to same-aged peers) demonstrates a robust association with depressive symptoms in girls (Graber et al., 1997; Hayward et al., 1997; Stattin and Magnusson, 1990). The evidence for boys is more mixed, with both delayed (e.g. Graber et al., 1997) and advanced gonadarche associated with elevated depressive symptoms (e.g. Kaltiala-Heino et al., 2003).

Conversely, a dearth of research has examined whether the timing of adrenarche predicts depressive symptoms (Byrne et al., 2016), despite evidence that 1) premature adrenarche (a clinical condition associated with significantly higher adrenal androgen concentrations) is associated with higher depressive symptoms in boys and girls (Dorn et al., 2009), and 2) there is evidence that the hormones associated with adrenarche are particularly relevant for understanding depression risk. For example, among children and adolescents, both high and low DHEA levels have been associated with mood disturbances in cross-sectional (Graber et al., 2006) and longitudinal samples (Goodyer et al., 2003, 2000). Additionally, in adolescent females, higher testosterone has been associated with higher depressive symptoms (Angold et al., 1999).

Mixed findings in the direction of relationships between adrenarcheal hormones and depression suggest that intermediary processes may mediate this link. Longitudinal research has demonstrated that structural brain changes may partly explain the relationship between early puberty and depressive symptoms. For example, we have previously found that larger pituitary volume mediates the relationship between pubertal timing and depressive symptoms in adolescents (Whittle et al., 2012). Although there is no similar research investigating brain structural mediators of adrenarcheal timing and depressive symptoms, adrenarcheal hormones are associated with structural brain development. For example, a large longitudinal study found positive associations between DHEA and cortical thickness in children and adolescents, and sexually dimorphic associations between testosterone and cortical thickness in pre-pubertal children (Nguyen et al., 2013). The structure of one region, the hippocampus, is particularly likely to be important in the link between adrenarcheal timing and depression, given links between androgens and hippocampal structure, and between hippocampal structure and depression.

A considerable body of animal research demonstrates that androgen receptors are abundant in the hippocampus (e.g. Beyenburg et al., 2000; Sarkey et al., 2008). Both testosterone and DHEA have been shown to exert neuroprotective and proliferative effects in the rodent hippocampus (Galea et al., 2006). Other research, however, suggests that elevated concentrations of androgens, such as DHEA, can have neurotoxic effects, resulting in neuronal loss in the hippocampus (Safiulina et al., 2006). This is supported by human research showing that elevated testosterone levels for one's age are associated with reduced hippocampal volumes in children and adolescents (see Herting et al. (2018), for a review). These findings suggest that although adrenarcheal androgens may be implicated in typical hippocampal development, exposure to particularly high concentrations may have deleterious effects on the hippocampus.

Further, altered hippocampal volumes are one of the most replicated findings in depressed individuals, with meta-analyses of adult major depression demonstrating moderate volumetric reductions compared to healthy individuals (Schmaal et al., 2016). Importantly, there is evidence that reduced hippocampal volumes are present before onset of illness and thus may represent a pre-existing vulnerability factor (Gilbertson et al., 2002), while recurrent illness may further reduce hippocampal volume (MacQueen and Frodl, 2011). Although the evidence base in youth samples is smaller, and results are more mixed (MacMillan et al., 2003; Rosso et al., 2005), some studies have found reduced hippocampal volumes in depressed children and adolescents (similar to the adult literature; (Caetano et al., 2007; MacMaster and Kusumakar, 2004). The mixed results of studies with young samples may be partly due to the confound of competing maturational processes, given that some samples spanned ages of 8–18 years, and hippocampal volumes typically increase during childhood and early to mid-adolescence (Herting et al., 2018; Wierenga et al., 2014). Age-restricted samples are therefore required to disentangle pathological and age-related developmental effects.

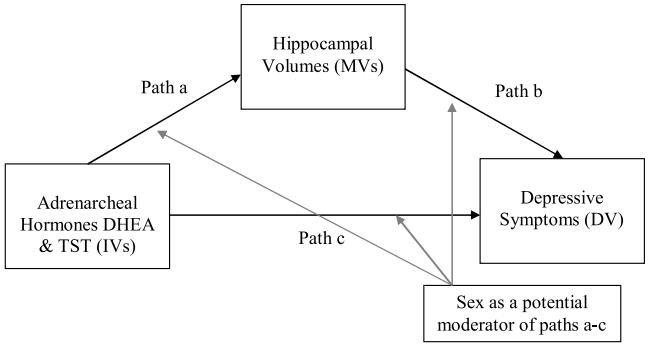

Fig. 1. Moderated mediation model examined.
Note. DHEA = Dehydroepiandrosterone; TST = Testosterone; IV = Independent variable; MV = Moderating variable; DV = Dependent variable.

The present study examined the cross-sectional relationships between adrenarcheal timing, hippocampal volumes, and depressive symptoms in a sample of children, aged 8–9 years. Specifically, it explored the extent to which hippocampal volumes mediated the relationship between adrenarcheal timing (as indicated by levels of the androgens DHEA and testosterone, controlling for age) and depressive symptoms, and whether sex moderated these relationships (the hypothesized model is depicted in Fig. 1). It was expected that hippocampal volumes would statistically mediate the association between adrenarcheal timing and depression in children, such that earlier timing (i.e., higher levels of DHEA and testosterone) would indirectly predict greater depressive symptoms via decreased hippocampal volumes. While the literature above inconsistently implicates testosterone versus DHEA, we hypothesized similar effects for both hormones primarily based on the literature on premature adrenarche, where this condition (associated with elevated levels of both hormones) is consistently associated with depression. In order to examine specificity of the results to the hippocampus, subsequent analyses also examined the relationship between adrenarcheal timing and whole-brain cortical structure (thickness, surface area and volume).

2. Method

2.1. Participants and recruitment procedure

This study utilized data from the 'Imaging brain development in the Childhood to Adolescence Transition Study' (iCATS; (Simmons et al., 2014), which is embedded in the larger 'Childhood to Adolescence Transition Study' (CATS; (Mundy et al., 2013), conducted by The University of Melbourne and the Murdoch Children's Research Institute (MCRI). Data for the current study was collected from a cross-sectional sample of 128 children in grade 3 (8–9 years) at intake (52% female, mean age 8.9 years, range 8.3–9.7 years), enrolled in 26 primary schools (19 Government, 2 Independent, and 5 Catholic) in metropolitan Melbourne, Australia.

Children participating in CATS were originally recruited through their primary schools. After parent/guardian consent was obtained, 1239 participants provided a saliva sample at their school between 9am and 10am. Saliva samples were analyzed for levels of DHEA and testosterone, and adrenarcheal development was modeled by plotting DHEA and testosterone levels for all participants. Equal numbers of

male and female children whose DHEA *and* testosterone levels fell in the upper or lower tertiles, relative to the cohort, were invited to participate in iCATS.

A total of 377 families were invited to take part in brain imaging, however 241 declined to participate mostly due to practical reasons (e.g., time). No significant differences in age, DHEA, testosterone or sex were observed between selected participants who consented or declined to participate in brain imaging (all p values > 0.05). A further eight participants were excluded based on a number of criteria: contra-indication for an MRI scanner (i.e., non-removable ferrous-based metals), head trauma history, clinically significant developmental/intellectual disorders, and current or past medication use that could have interacted with their endocrine system (i.e., steroid or amphetamine based drugs).

One hundred and twenty-eight participants underwent a structural brain scan at the Royal Children's Hospital (RCH) approximately 7 months after the baseline assessment (M age = 9.5 years, SD = 0.4). Two further saliva samples were collected to obtain a more proximal measure of adrenarcheal development. At the time of the scan, parents also completed the Sexual Maturity Scale – Parent Report (SMS-PR) and children completed the Children's Depression Inventory – Second Edition (CDI-2). After visual inspection of the brain scans, six participants were excluded due to movement artefacts and a further 34 participants did not provide adequate saliva samples or were missing survey data, leaving a final sample of 88 participants (42 male, 46 female) for the analyses. None of the participants were diagnosed with premature adrenarche or precocious puberty, based on parent report. Written consent was obtained from the parent/guardian and verbal assent from the child. Ethics approval was granted by the Royal Children's Hospital Human Research Ethics Committee (#32,171). Note that adrenacheal data of this sample have previously been reported in relation to white matter volume (Klauser et al., 2015), white matter integrity (Barendse et al., 2018b), affective brain function (Whittle et al., 2015) and connectivity (Barendse et al., 2018a).

2.2. Design and measures

2.2.1. MRI data acquisition & preprocessing

Structural neuroimaging data were acquired by a 3 T Siemens TIM Trio scanner at the Murdoch Children's Research Institute (MCRI), RCH, Melbourne. Participants lay supine with their head supported in a 32-channel head coil. T1-weighted images were acquired during a 3.5 min sequence (repetition time = 1900 msec; echo time = 2.24 msec; flip angle = 9°, field of view = 23 cm^2), which produced 208 0.9 mm contiguous sagittal slices (voxel dimensions = 0.9 mm^3).

Images were transferred to an SGI/Linux workstation for morphometric analysis. FreeSurfer Version 5.3 software was used to estimate hippocampal and whole brain volumes (http://surfer.nmr.mgh.harvard.edu/). Manual corrections to the gray matter-white matter surface were made to the majority of images and were performed by AF. No manual editing to the subcortical segmentation was deemed necessary. Whole brain volume (WBV) was calculated as the total of all gray and white matter from the FreeSurfer segmentation, and hippocampal volumes were corrected for WBV to decrease the risk of observing structural difference between individuals solely due to variation in head or body size. These corrected hippocampal volume estimates were used in all subsequent analyses.

2.2.2. Saliva samples

Children collected a saliva sample with the help of their parent/guardian on the day of, and day prior to the MRI scan. As DHEA and testosterone exhibit diurnal rhythms, with highest values in the morning, participants were asked to collect their samples immediately after waking (Ankarberg and Norjavaara, 1999). Samples were initially frozen at −30 °C, and prior to analysis, defrosted and centrifuged, with the supernatant assayed in duplicate for levels of DHEA and testosterone using Salimetrics ELISA kits. Salivary assays of these hormones have been shown to correlate well with serum levels, and an average of the two hormone measurements was used in analyses (Granger et al., 2004, 1999). Individuals below the limit of detection (LOD) of DHEA (5 pg/ml) were assigned a value of 2.5, which was halfway between zero and the detectable limit, an approach consistent with previous research (Cohen and Ryan, 1989). This was the case for nine individuals. There were no individuals with testosterone values below the LOD (1 pg/ml). The inter-assay coefficients of variation (CVs) for DHEA and testosterone were 5.45% and 13.54%, respectively, and intra-assay CVs 8.56% and 7.32%, respectively.

2.2.3. Sexual maturity status parent report (SMS-PR)

The SMS-PR comprises a series of line drawings of girls and boys bodies, based on the five Tanner stages of physical pubertal development (Marshall and Tanner, 1970, 1969). Parents are presented with five images of male or female pubic hair development, and asked to circle the image that most accurately represents their child's development. Parents with a female child complete an additional item with five stages of breast development. These ratings have been shown to correlate well with physical examinations (Dorn and Biro, 2011). For girls, where pubic hair and breast development ratings differed, the higher value was used in the analysis. SMS-PR scores are henceforth referred to as Tanner stages.

2.2.4. Children's depression inventory (CDI-2)

The CDI-2 is a self-rated questionnaire for children and adolescents aged 7–17 years designed to measure depressive symptoms over the past two weeks. The CDI-2 has been found to be a valid and reliable measure for use in child community samples (Kovacs, 2004). It contains 28 items assessing symptoms across four domains: negative mood/physical symptoms, negative self-esteem, ineffectiveness, and interpersonal problems. Item scores range from 0 to 2 (0 = absence of symptoms, 1 = mild or probable symptom, 2 = definite symptom), and half of the item choices are listed in reverse order. These items are used to generate two subscale scores (emotional and functional problems), which sum to produce a total score ranging from 0-56, with higher scores reflecting more severe and frequent depressive symptoms. Total raw scores can be converted to T-scores by sex and age range (7–12 years or 13–17 years), with T-scores of 65 and above indicating elevated depressive symptoms. T-scores were used in analyses. The majority of children in the current sample reported low levels of depressive symptoms, with 93% (38 males and 44 females) obtaining a T-score $<$ 65, while 7% (4 males and 2 females) obtained a T-score of $\geq$ 65.

2.2.5. Body mass index (BMI)

Two measures of height (to nearest 0.1 cm) and weight (to nearest 0.1 kg) were obtained and averaged, and used to calculate a body mass index (BMI) for each participant.

2.3. Statistical analyses

For hippocampal volumes, correlations and t-tests were performed using the IBM Statistical Package for the Social Sciences (SPSS) Version 22. Alpha was set at $p <$ .05. Missing data estimation, hypothesis testing, and mediation analyses were conducted using M*plus* software Version 6.12 (Muthén and Muthén, 2011). Adrenarcheal timing was operationalized as levels of DHEA and testosterone, controlling for age. Continuous measures of the hormones were used here (concurrent with other assessment), instead of the baseline tertile groups used for recruitment, to be able to examine the hormones separately, and to obtain a more refined picture of the association of each with hippocampal volume and depressive symptoms. DHEA and testosterone were log transformed due to their non-normal distribution. Separate mediation analyses were conducted for DHEA and testosterone by hemisphere (4 analyses total) because these hormones demonstrate high correlations,

as do hippocampal volumes in each hemisphere. The effect of age and Tanner stage on depressive symptoms was controlled for in each analysis in order to rule out the possibility that increases in depressive symptoms were due to overt physical signs of adrenarche or the early onset of gonadarche. Given BMI may be associated with pubertal timing, a sensitivity analysis was conducted to assess whether the inclusion of BMI had any effect on significant findings.

Whole brain cortical analyses were conducted using the FreeSurfer image analysis suite GLM Analysis ("mri_glmfit"), where cortical thickness/surface area/volume was regressed on each of the hormones, controlling for age and moderated by sex. A cluster-forming threshold was set at $p < 0.001$ and resulting maps were then cluster-wise corrected for multiple comparisons ($p < 0.05$) with pre-computed Monte-Carlo simulations of 10,000 iterations that are provided with the FreeSurfer suite ("mri_glmfit-sim"). Thickness, surface area or volume for any significant clusters identified were extracted and subject to mediation analyses, as described for hippocampal volumes.

2.3.1. Approaches to statistical mediation analysis

The hypothesized mediation model (Fig. 1) was tested with path analysis, a form of Structural Equation Modelling (SEM). It estimates the relative strengths of both direct and indirect relationships among a set of variables, and is recommended for mediation models as it provides measures of global fit. This approach permits the comparison of nested models (i.e., where the hypothesized relationships may vary by sex). The adequacy of overall model fit was assessed based on the consideration of the model chi-square (χ^2), the comparative fit index (CFI), the root mean square error of approximation (RMSEA), and the standardized root mean squared residual (SRMSR). The fit cut-off values for these indices, utilized in the current study, have been recommended for continuous data and models employing maximum likelihood (ML; (Hu and Bentler, 1999).

The statistical significance of direct effects (paths a and b in Fig. 1) and the total effect (path c, Fig. 1) was determined on the basis of a $p <$ .05 and a 95% confidence interval (CI) for a parameter estimate that did not pass through zero. A bias-corrected bootstrapping method was applied to test the significance of the mediation (or indirect, a*b) effects (the cross product of paths a and b; Fig. 1) (Hayes, 2009). As recommended (Hayes, 2009), 5000 resamples were taken in the present analyses and 95% confidence intervals that did not pass through zero were considered evidence of mediation.

2.3.2. Testing for sex effects

Multiple group analyses were employed to test the potential moderating effect of sex on the hypothesized relationships. Initially, parameter estimates for the model paths (i.e., total, direct and indirect paths a–c) were constrained to be equal across groups to test the null hypothesis that there were no sex differences in the modeled relationships. The modification indices (MIs) produced by *Mplus* were then examined to determine the measurement invariance across the two sexes. Following the procedure recommended by (Muthén and Muthén, 2007), if a constrained parameter had a MI of > 3 in both male and female groups, and releasing it could be justified theoretically, then the parameter was released and the model was re-run. A chi-square difference test was employed to determine whether releasing the identified parameter resulted in a significant improvement in model fit. A significant chi-square difference test indicates that constraining the parameter to be equal across the sexes significantly worsens model fit, suggestive of a significant sex difference for that parameter. Where multiple parameters indicated a MI of > 3, one parameter was released at a time, starting with the parameter with the highest MI. Subsequent parameters were released following the same procedure as above until the best fitting model was identified.

Table 1
Means and Standard Deviations of Age, Salivary Hormones, Depressive Symptoms and Tanner Stage.

Variable	Males ($N = 42$)	Females ($N = 46$)	Total	t
Age (years)*	9.6 (0.4)	9.4 (0.3)	9.5 (0.3)	2.6
DHEA*	41.9 (30.7)	73.6 (99.0)	58.4 (76.0)	−2.1
TST	30.2 (9.6)	32.9 (15.4)	31.6 (12.9)	−.98
L Hippocampus*	4553.0 (325.0)	4393.8 (316.9)	4469.8 (328.8)	2.3
R Hippocampus	4313.8 (323.8)	4217.1 (281.0)	4263.3 (304.4)	1.5
CDI	49.6 (9.1)	50.5 (28.7)	50.1 (8.9)	−.47
Tanner	1.3 (0.4)	1.2 (0.4)	1.2 (0.4)	0.6
BMI*	17.1 (2.0)	18.2 (2.6)	17.7 (2.4)	−2.1

Note. DHEA = dehydroepiandrosterone; TST = testosterone; L = Left; R = Right; CDI = Children's Depression Inventory; Tanner = Tanner stage. Hippocampal volumes are corrected for whole brain volume by sex.
* $p < .05$.

3. Results

3.1. Analysis of descriptive data and sex differences

The means, standard deviations, and sex differences among key variables are presented in Table 1. Independent samples t-tests showed that males were significantly older than females at the time of the MRI scan and had significantly larger WBV-corrected left hippocampal volumes. Girls, however, had significantly higher levels of DHEA. Supplementary Table S1 presents bivariate correlations between variables separately for males and females. Supplementary Figure S1 presents histograms of key variables used in analyses.

3.2. Hypothesis testing using path analysis

3.2.1. Testing overall model fit

Supplementary Table S2 presents the model fit indices for each path analysis. The chi-square test was not significant at the 5% level for all four models, indicating good fit to the data. The values for the CFI, RMSEA, and SRMSR differed somewhat between models, indicating that the DHEA models fit the data better than the testosterone models.

3.2.2. Sex moderation

Regarding the total effect (path c, relationships between adrenarcheal hormones and depressive symptoms, controlling for age and Tanner stage), multiple group models showed that sex significantly moderated the association between testosterone and depressive symptoms only. The equality constraints on two regression paths were released consecutively, resulting in significant improvements in the model fit based on the traditional chi-square difference test; testosterone regressed on CDI (MI = 4.53), $\chi^2_{diff}(1) = 4.78$, $p < .05$, and Tanner stage regressed on CDI (MI = 4.01), $\chi^2_{diff}(1) = 4.08$, $p < .05$.

Multiple group analyses were conducted to evaluate whether sex moderated the direct (a and b) and indirect (a*b) relationships proposed. The equality constraint on the same direct regression path was released for both hemispheres of the DHEA model based on large MIs and significant improvements in model fit; age to CDI (MI = 4.54), $\chi^2_{diff}(1) = 4.79$, $p < .05$, and age to CDI (MI = 4.17), $\chi^2_{diff}(1) = 4.29$, $p < .05$ for the left and right hemispheres, respectively. The equality constraints on two different direct regression paths were released consecutively for each hemisphere of the testosterone models based on large MIs and significant improvements in model fit; left hippocampus regressed on testosterone (MI = 7.14), $\chi^2_{diff}(1) = 7.60$, $p < .01$, and age regressed on CDI (MI = 4.54), $\chi^2_{diff}(1) = 4.79$, $p < .05$ for the left hippocampus; and testosterone regressed on the right hippocampus (MI = 4.68) $\chi^2_{diff}(1) = 4.87$, $p < .05$; and age regressed on CDI (MI = 4.13), $\chi^2_{diff}(1) = 4.26$, $p > .05$ for the right hippocampus. These modifications resulted in changes in the significance of the regression paths implicated, demonstrating that sex moderated several

Table 2
Parameter Estimates for Direct Effects in the Tested Model (DHEA and the Left Hippocampus).

	Standardized Estimates		95% Confidence Interval (CI)		
	β	SE β	Lower 2.5%	Upper 2.5%	*p*
Left hippocampus regressed on					
DHEA	.22/.35	.08/.12	**.07/.11**	**.38/.59**	**.01/.00**
Age	−.20/−.19	.11/.11	−.41/−.40	.01/.02	.06/.07
CDI regressed on					
DHEA	−.00/−.00	.08/.13	−.16/−.25	.16/.24	.98/.98
Left hippocampus	.23/.23	.10/.11	**.02/.02**	**.43/.44**	**.03/.03**
Age	−.04/.40	.15/.13	−.34/**.15**	.25/**.66**	.78/**.00**
Tanner stage	−.17/−.21	.10/.11	−.36/−.43	.02/.01	.08/.06

Note. β = Standardized regression parameter estimate; SE = Standard error; DHEA = Dehydroepiandrosterone; CDI = Children's Depression Inventory. β, SE β and 95% CI for standardized estimates reported as males/females; Statistically significant confidence intervals (i.e. excluding zero) are highlighted in bold font. *p* = two-tailed.

but not all of the direct relationships between adrenarcheal hormones, hippocampal volumes, and depressive symptoms.

3.2.3. Total effects

DHEA did not significantly predict CDI scores for boys or girls. For boys, however, lower levels of testosterone significantly predicted higher CDI scores, β = -.31, p = .03, 95% CI: (-.58, -.03), as did lower Tanner scores, β = -.43, p = .001, 95% CI: (-.69, -.16), accounting for 22% of the variance in CDI scores.

3.3. Direct effects

All standardized regression parameter estimates, standard errors of parameter estimates, 95% CIs, and two-tailed *p*-values for the a and b paths of the four models are presented in Tables 2–5.

DHEA demonstrated a significant positive association with the volume of the left hippocampus in males and females, and testosterone demonstrated a significant positive association with left and right hippocampi in females only. Testosterone did not significantly predict hippocampal volumes in males. Larger left and right hippocampi significantly predicted higher CDI scores for males and females.

3.3.1. Indirect effects

All unstandardized regression parameter estimates, standard errors, and bootstrapped 95% CIs for the indirect paths are presented in Supplementary Table S3. Analyses demonstrated a significant positive indirect effect from testosterone to CDI via larger right hippocampi for girls (effect = 1.29, SD = 0.90, CI = 0.11-3.78), accounting for 20% of the variance in CDI scores. No other significant indirect effects were observed.

Summaries of the significant total, direct and indirect relationships observed in the models for DHEA and testosterone are presented in Supplementary Figures S2-4. Scatterplots of all significant associations described in models are presented in Supplementary Figure 5. Notably, in sensitivity analyses including BMI in the models, all significant results retained significance.

3.3.2. Cortical analysis

The regression analyses of cortical thickness, surface area and volume on hormone levels did not reveal any significant clusters. As such, mediation was not indicated nor tested.

3.3.3. Interaction analysis

Given evidence that the interaction of androgen hormones may impact on neurodevelopment and mood (Goodyer et al., 1996; Nguyen et al., 2013), and some evidence that testosterone and DHEA interact to predict neurodevelopment (Nguyen et al., 2013), subsequent analyses also checked for interaction effects of DHEA and testosterone on hippocampal volume and depressive symptoms. No associations were found to be significant (all p values > 0.05).

4. Discussion

The results of this investigation partially supported the hypothesized relationships between adrenarcheal timing, hippocampal volumes, and depression, though results were in the opposite direction to those expected, and varied by hormonal indicator and sex. Although we did not make explicit hypotheses about associations between adrenarcheal hormone levels and depressive symptoms, it was notable that we found that late adrenarcheal timing, as suggested by lower levels of

Table 3
Parameter Estimates for Direct Effects in the Tested Model (DHEA and the Right Hippocampus).

	Standardized Estimates		95% Confidence Interval (CI)		
	β	SE β	Lower 2.5%	Upper 2.5%	*p*
Right hippocampus regressed on					
DHEA	.08/.13	.08/.13	−.08/−.12	.24/.38	.31/.32
Age	−.17/−.17	.11/.11	−.38/−.39	.04/.04	.10/.11
CDI regressed on					
DHEA	.02/.04	.08/.12	−.12/−.20	.17/.28	.75/.75
Right hippocampus	.26/.26	.10/.10	**.06/.06**	**.45/.47**	**.01/.01**
Age	−.03/.40	.15/.13	−.32/**.15**	.27/**.65**	.86/**.00**
Tanner stage	−.14/−.18	.09/.11	−.29/−.41	.04/.04	.13/.13

Note. β = Standardized regression parameter estimate; SE = Standard error; DHEA = Dehydroepiandrosterone; CDI = Children's Depression Inventory. β, SE β and 95% CI for standardized estimates reported as males/females; Statistically significant confidence intervals (i.e. excluding zero) are highlighted in bold font. *p* = two-tailed.

Table 4
Parameter Estimates for Direct Effects in the Tested Model (Testosterone and the Left Hippocampus).

	Standardized Estimates		95% Confidence Interval (CI)		
	β	SE β	Lower 2.5%	Upper 2.5%	*p*
Left hippocampus regressed on					
TST	−.18/.46	.15/.12	−.47/**.23**	.12/**.69**	.23/**.00**
Age	−.14/−.13	.11/.10	−.35/−.32	.07/.07	.19/.22
CDI regressed on					
TST	.00/.00	.08/.12	−.15/−.24	.16/.24	.98/.98
Left hippocampus	.22/.23	.10/.11	**.02/.02**	**.41/.44**	**.03/.03**
Age	−.04/.40	.15/.13	−.34/**.15**	.25/**.65**	.77/**.00**
Tanner stage	−.17/−.21	.10/.11	−.35/−.43	.02/.02	.08/.07

Note. β = Standardized regression parameter estimate; SE = Standard error; TST = Testosterone; CDI = Children's Depression Inventory. β, SE β and 95% CI for standardized estimates reported as males/females; Statistically significant confidence intervals (i.e. excluding zero) are highlighted in bold font. *p* = two-tailed.

testosterone, was significantly associated with higher levels of depressive symptoms in boys, while no significant relationships were observed in girls. Contrary to predictions, early adrenarche, as indicated by higher levels of DHEA, predicted *larger* left hippocampal volumes in the entire sample, and early adrenarche, as indicated by higher testosterone levels, predicted larger bilateral hippocampal volumes in girls only. In turn, larger bilateral hippocampal volumes were associated with significantly higher levels of depressive symptoms in boys and girls. Finally, a significant indirect or mediatory effect was observed for girls such that early timing as indicated by higher testosterone was related to increased depressive symptoms via larger right hippocampal volumes.

Late timing (indicated by lower levels of testosterone and less advanced Tanner stage) was related to higher levels of depressive symptoms in boys. Although the evidence for associations between adrenarcheal timing and depression is mixed for boys, this finding is consistent with evidence that delayed pubertal development can confer risk for depression (Alsaker, 1992; Graber et al., 1997). The maturational deviance hypothesis, which suggests that any deviation from typical development is stressful and therefore increases risk for mood problems, may provide a partial explanation for the present findings (Petersen and Taylor, 1980). However, as the physical changes of adrenarche are less prominent compared to those associated with subsequent gonadal changes, explanations based on hormonal mechanisms may be more informative. The present finding that lower testosterone levels were associated with increased depressive symptoms in boys supports research demonstrating that testosterone generally confers resiliency against depression. For example, hypogonadal men (those with deficient testosterone) are most susceptible to depression (Shores et al., 2004), and in some cases testosterone administration can reduce depressive symptoms (Seidman and Rabkin, 1998). Longitudinal studies with children are required to examine the developmental implications of these findings for boys.

Although there was no direct effect of adrenarcheal timing on depressive symptoms for girls, a significant indirect or mediatory role of the right hippocampus was found, though the directions of associations were unexpected. Specifically, earlier adrenarcheal timing (indicated by higher testosterone levels) was associated with greater levels of depressive symptoms via larger right hippocampal volumes in girls. Rodent research suggests that testosterone may have proliferative, neurogenic effects in the hippocampus (Galea et al., 2006), and as such this may have been the case for girls in this study. Although such an effect has been described as neuroprotective, given the links with depressive symptoms found in the present study (and discussed below), other interpretations may be warranted.

Some studies have shown volumetric hippocampal reductions in clinically depressed individuals, particularly those with chronic depression (Schmaal et al., 2016), although there is some evidence that smaller volumes may represent a pre-existing vulnerability factor (Gilbertson et al., 2002). Our finding that larger hippocampal volumes were associated with higher levels of depressive symptoms was thus unexpected. However, it is consistent with some reports of hippocampal enlargements in adolescents and adults with major depression compared to controls, particularly in those experiencing their first depressive episode (Frodl et al., 2002). Also, the majority of children in the present sample reported subclinical levels of depressive symptoms, which may be why our results differ to those in clinically depressed individuals. An association between larger hippocampi and increased depressive symptoms may be explained by the critical role of the hippocampus in feedback inhibition of the HPA axis (a network that typically becomes activated in response to stress). Enlarged hippocampal volumes, partly caused by early exposure to testosterone in females, may signal increased attempts to regulate stress-related HPA axis activity, associated with inflated depressive symptoms (Herman et al., 2005). An alternate (although not mutually exclusive) explanation

Table 5
Parameter Estimates for Direct Effects in the Tested Model (Testosterone and the Right Hippocampus).

	Standardized Estimates		95% Confidence Interval (CI)		
	β	SE β	Lower 2.5%	Upper 2.5%	*p*
Right hippocampus regressed on					
TST	−.18/.34	.15/.13	−.47/**.08**	.12/**.59**	.24/**.01**
Age	−.15/−.15	.10/.11	−.35/−.35	.05/.02	.14/.15
CDI regressed on					
TST	.01/.02	.08/.12	−.13/−.21	.16/.22	.86/.86
Right hippocampus	.26/.27	.10/.11	**.07/.06**	**.45/.44**	**.01/.01**
Age	−.02/.40	.15/.13	−.31/**.15**	.27/**.61**	.88/**.00**
Tanner stage	−.13/−.17	.09/.11	−.31/−.39	.05/.01	.15/.13

Note. β = Standardized regression parameter estimate; SE = Standard error; TST = Testosterone; CDI = Children's Depression Inventory. β, SE β and 95% CI for standardized estimates reported as males/females; Statistically significant confidence intervals (i.e. excluding zero) are highlighted in bold font. *p* = two-tailed.

comes from the role of the hippocampus in fear processing. For example, (Mueller et al., 2009) found that compared to controls, males with hypergonadism (a medical condition of excessive testosterone) showed elevated hippocampal fear processing as well as faster behavioral responses to faces showing fearful expressions. Although these findings have not been confirmed in females, they suggest that high levels of testosterone may be associated with hippocampal hypersensitivity to fear, which may in turn lead to depressive behaviors like avoidance and withdrawal.

The sex differences in findings are notable. Higher levels of testosterone were associated with larger bilateral hippocampi (and with depressive symptoms via hippocampal volume) for girls only. As boys demonstrated significantly lower levels of DHEA despite being significantly older than girls in the present sample, this suggests that adrenarcheal processes had not been underway for the same duration as girls, which may explain the absence of a significant mediatory effect. Sex differences in hippocampal volumes have previously been observed during later phases of puberty (Hu et al., 2013; Satterthwaite et al., 2014). The precise mechanisms through which testosterone impacts the hippocampus in a sex-dependent manner are likely complex, and require further investigation.

Also of note was that, similar to testosterone (although for both sexes, not only girls), there was an association between higher levels of DHEA and larger (left) hippocampal volumes. This finding is consistent with animal research demonstrating neuroprotective and proliferative effects of DHEA in the hippocampus (Galea et al., 2006; Maninger et al., 2009). Unlike testosterone, however, there was no indirect effect of DHEA on depressive symptoms. Although DHEA and testosterone were highly correlated, they are separate hormones and their effects on brain, development and behavior likely differ. The present results suggest that testosterone and DHEA should be investigated separately in relation to questions about adrenarcheal timing, neurodevelopment and mental health.

This study did not find any effect of adrenarcheal hormone levels on cortical structure in a whole-brain cortical analysis. This is in contrast with previous research; namely, (Nguyen et al., 2013) reported associations between DHEA and development of thickness of several cortical areas. Their analysis, however, was longitudinal (in individuals aged 4–22 years) and was not designed to tap into adrenarcheal timing per se. Our finding reinforces the importance of the hippocampus as a brain region that is specifically involved in the relationship between adrenarcheal timing and depression.

5. Limitations

Several limitations of this study should be addressed. First, its cross-sectional design limits the capacity for directional relationships to be inferred, and causality cannot be established. As pubertal timing is influenced by early psychosocial stress, differences in hippocampal volumes and depressive symptoms in the present sample may be the result of early adverse experiences (e.g., trauma, family conflict, socio-economic disadvantage) rather than adrenarcheal hormones alone (Mensah et al., 2013; Tupler and De Bellis, 2006). Further, there is an emerging literature base documenting longitudinal associations between pubertal hormones and hippocampal structure (Vijayakumar et al., 2018), and such research is important for teasing apart the effects of timing and tempo of hormonal exposure on brain development. Therefore, longitudinal research is needed that begins prior to adrenarche, and captures environmental exposures in addition to longitudinal measures of hormones, hippocampal volume and depressive symptoms. Such research may clarify the extent to which the timing of adrenarcheal hormone exposure influences brain development and uniquely predicts the onset and course of depression.

Although salivary hormone levels were collected twice in the morning to obtain their peak aggregate, adrenarcheal hormones display variation throughout the day and across time, therefore, assessing prolonged hormone exposure using measures such as hair samples could provide more robust estimates of long-term hormone exposure (Sauvé et al., 2007). Also, it is possible that variation in relative exposure to androgen levels may not only reflect differences in the timing of adrenarche, but may also indicate individual differences in absolute hormones levels. Further, although Tanner stage was controlled for to account for early gonadarche, we cannot rule out that testosterone levels were not influenced by hypothalamic-pituitary-gonadal axis function.

While the current study did not find effects of the interaction between DHEA and testosterone on hippocampal volumes or depressive symptoms, interactions with other hormones may be important. In particular, high cortisol/low DHEA ratios have been found in cases of persistent major depression in adolescents (Goodyer et al., 2003). Therefore, examining the interactive effects of cortisol and adrenal hormones in future may help elucidate their impact on brain development and mood.

Despite these limitations, the present study has a number of strengths, addressing some important gaps in the literature. Primarily, this study examined the impact of adrenarcheal timing on depressive symptoms in a non-clinical sample of children, including boys and girls in similar proportions. Similarly, while previous studies have confounded aspects of pubertal development such as age, timing, and pubertal stage, the inclusion of adrenarcheal hormones and Tanner stage in an age-restricted sample allowed their relative effects on depressive symptoms to be delineated. Furthermore, by examining the impact of hippocampal volumes on depressive symptoms during adrenarche, the present study integrated trends identified in both the hormone and depression literatures, identifying a potential mechanism through which adrenarcheal timing may influence mood.

6. Conclusion

The early identification of risk factors for depression is imperative for the development of treatment interventions that may help to reduce its incidence and burden. The findings of the current study suggest that the relative timing of adrenarcheal hormone exposure and hippocampal development are differentially associated with depressive symptoms in boys and girls during late childhood. While longitudinal designs are needed to clarify the sequence of relationships, the present results considerably advance understandings of depression risk processes, and may have implications for the development of interventions targeting pubertal adjustment.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.02.016.

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