# Pubertal hormones and brain structure: Exploring the value of hair assays

Nandita Vijayakumar[ab], Elizabeth A. Shirtcliff[c], Michelle L. Byrne[a], Kathryn L. Mills[a], Theresa W. Cheng[a], Arian Mobasser[a], Jessica E. Flannery[a], Benjamin W. Nelson[a], John C. Flournoy[d], Wen Wang[c], Nicholas B. Allen[a], Jennifer H. Pfeifer[a]

[a] Department of Psychology, University of Oregon, Oregon, USA

[b] School of Psychology, Deakin University, Melbourne, Australia

[c] Human Development Family Studies, Iowa State University, Iowa, USA

[d] Department of Psychology, Harvard University, Cambridge, USA

**Corresponding Author:**
Nandita Vijayakumar
School of Psychology
Deakin University
221 Burwood Hwy
Burwood, VIC, Australia
Ph: +61447074979
nvijaya@deakin.edu.au

**Abstract**

Neuroimaging research has highlighted the role of puberty in structural brain development in humans, but studies investigating the mechanistic role of hormones in this association have produced inconsistent findings. Limitations of current approaches to hormonal assessments have long been recognized, as basal hormone levels are susceptible to momentary influences (in particular, circadian rhythmicity and menstrual cyclicity). However, emerging research suggests that a novel method of assaying pubertal hormone concentrations in hair may overcome some of these issues by capturing hormonal exposure across a longer period of time. This study is the first to compare associations between hormone concentrations measured via hair and saliva with brain structure in a sample of early adolescent females (N = 112, 10-13 years of age). Estradiol, testosterone, and DHEA concentrations were assayed from i) 5cm hair samples collected proximal to the scalp, reflecting approximately 5 months of hormonal exposure, and ii) repeated weekly saliva samples collected over the course of one month. Participants also underwent structural MRI scans, and estimates of cortical thickness and subcortical volume were obtained. Findings revealed that pubertal hormones in saliva samples exhibited strongest associations with parieto-occipital cortices. Comparatively, hair hormone concentrations exhibited stronger negative associations with cingulate and lateral prefrontal cortical thickness, which may reflect unique developmental processes that occur across longer periods of hormonal exposure. However, controlling for pubertal stage removed much of the cortical associations with hormones in saliva, and resulted in minimal change in cortical associations with hormones in hair. Thus hormone concentrations in hair may reflect biological processes not captured by self-reported pubertal stage that influence brain development. Further research is needed to improve our understanding of these potentially unique neurodevelopmental processes captured by saliva and hair hormone concentrations.

**Key words:** puberty; saliva hormones; hair hormones; brain structure; magnetic resonance imaging

## 1. Introduction

Puberty is characterized by physical, neural and biological changes that support the process of sexual maturation. Hormonal shifts result in increased growth and metabolic rate, alterations in fat and muscle, development of breasts and genitalia, and the appearance of secondary sex characteristics in the body. Human and animal work shows pubertal hormones may also play a role in the maturation of neurobiological systems during adolescence (for reviews, see Goddings et al., 2019; Herting et al., 2017; Vijayakumar et al., 2018). While early theories proposed that only "activational" effects (i.e., temporary changes in brain activity) were present during puberty, emerging animal and human research from the past decade suggests that puberty is a period when hormones exert "re-organizational" effects on brain structure (Schulz, Molenda-Figueira, & Sisk, 2009).

Neuroimaging research indicates a general pattern of reductions in cortical grey matter thickness with pubertal development, while associations of puberty with subcortical structure are more inconsistent (for review, see Vijayakumar et al., 2018). The majority of studies have examined associations with pubertal stage – the five stages of physical development based on changes in secondary sex characteristics (pubic hair and genital/breast development for boys and girls, Tanner, 1962). A smaller set of studies have examined relationships between brain structure and pubertal hormones, but results are more mixed, including a number of null effects. For example, only three out of seven studies identified significant associations between testosterone concentrations and subcortical volume (Bramen et al., 2011; Herting et al., 2014; Neufang et al., 2009). Moreover, only two of the six studies on estradiol concentrations identified significant associations with subcortical structure, both of which focused on pituitary volume (Peper et al., 2010; Wong et al., 2014). These null effects contradict animal research that highlights the role of sex steroids on hormone receptors in the subcortex (Schulz et al., 2009; Sisk et al., 2017).

This begs the question of what factors may be contributing to the inconsistencies in pubertal neuroimaging research. Aside from pubertal maturation, hormone concentrations are influenced by a number of endogenous and exogenous factors. In post-menarcheal females, hormone levels significantly fluctuate over the menstrual cycle, with a recent study finding that individual variation across a menstrual cycle accounted for 59% of variability (Wang et al., 2019). Some studies collect biological samples within the early follicular phase during menses, and a smaller subset conduct frequent and systematic measurements across the menstrual cycle. However, anovulation and irregular menstrual cycles are particularly common in early adolescent females, and hormones often fluctuate in the years preceding menarche (Apter et al., 1978; Vihko & Apter, 1985). Calculation of "basal" or average hormone concentrations through repeated sampling over the course of a month may help overcome variance in hormone concentrations attributable to menstrual cyclicity, but such designs have not been utilized in neuroimaging research.

Another important consideration for pubertal neuroimaging research is the time frame over which endocrine changes impact the structure of cortical and subcortical regions. This relationship likely occurs over the course of months to years, with hormones influencing dendritic arborization and synaptic pruning, apoptosis and neurogenesis, as well as receptor density, in a region-specific manner (for review, see Juraska & Willing, 2017). However, issues with participant compliance makes it difficult to utilize current methods (i.e., saliva, blood or urine) to measure hormone concentrations over long time frames. The novel method of measuring pubertal hormone concentrations in hair samples may overcome this problem, as it can generate a "basal" metric reflecting months of cumulative hormonal exposure with minimal participant burden or non-compliance concerns. Indeed, hair samples have proven to be valuable for measuring chronic (i.e., cumulative) cortisol concentrations, providing an index of hormone concentrations over the course of months, depending on the length of hair

assayed (Stalder & Kirschbaum, 2012). Steroids diffuse into hair follicles, and similar to saliva samples, reflect unbound or "active" hormone levels that are free to act on receptors, including those within the brain. Emerging research is employing this methodology to examine pubertal hormone concentrations in humans (Smith et al., in press) and have shown that hair testosterone and DHEA concentrations are more stable "averages" than saliva estimates in young adult females (Wang et al., 2018). Recent studies have also shown that hair testosterone concentrations are related to psychopathology (Dettenborn et al., 2016), with one study finding that they exhibited a stronger association to externalizing problems than saliva hormone concentrations (Grotzinger et al., 2018). Given that neurobiology has been implicated in adolescent-onset psychopathology (for review, see Jones et al., 2017), it is critical to next investigate associations between hair hormone concentrations and brain structure.

### 1.2 Current study

This investigation addresses limitations in the structural neuroimaging literature by examining the relationship between multiple pubertal indices and brain structure in a sample of early adolescent females (aged 10.0 to 13.0 years). We utilized two methods of assessing pubertal hormones: "basal" saliva and hair measurements. "Basal" saliva measurements were based on an average of four saliva samples collected weekly over one month, in order to overcome issues relating to menstrual cyclicity. Hair hormone concentrations were averaged across the 5cm closest to the scalp, thus referencing approximately the prior 5 months, in order to index a longer period of cumulative hormonal exposure than saliva measurements. We also characterized puberty-brain associations across different physical and hormonal indices that relate to adrenarche and gonadarche, including estradiol, testosterone and DHEA concentrations.

Varied analytic strategies employed in prior research makes it difficult to always identify consistent patterns of associations. As such, we felt that a comprehensive characterization of effects sizes was warranted. Moreover, given the novelty of hair hormone investigations in neuroimaging research, this exploratory study predominantly focuses on correlation coefficients. Specifically, we describe associations between cortical and subcortical structure with hormone concentrations in basal saliva and hair measurements. We also examine whether hormone concentrations from hair explain additional variance in brain structure, over and above those from saliva. Given the methodological differences of hair compared to saliva samples, and in particular the differences in time frames indexed by the two measures, we hypothesized that hair hormone concentrations would be uniquely associated with cortical and subcortical structure. Finally, we explore associations between hormone concentrations and brain structure when controlling for pubertal stage, in order to understand whether similar developmental processes are indexed by hormonal and observational measures of puberty.

## 2. Method

### 2.1 Participants

A community sample of 174 adolescent girls, aged 10.0 to 13.0 years (mean = 11.55 years, SD = 0.81 years), were recruited from Lane County, Oregon, into a longitudinal project called Transitions in Adolescent Girls (TAG). The majority of participants were recruited from elementary and middle schools in the region, and a small subset were recruited by other methods (such as advertising at science fairs and Craigslist). Inclusionary criteria consisted of i) fluency in English, ii) no developmental disabilities (aside from Attention Deficit/Hyperactivity Disorders), iii) no diagnosis of psychotic disorders, iv) no MRI

contraindications, and v) an upper age limit of 13 years at entry into the study. The race and ethnicity of participants are presented in Table S1.

Of this larger sample, 10 elected not to participate in the MRI and a further 52 were excluded for poor quality structural MRI (see below for further detail). The final sample in this study consisted of the remaining 112 participants.

### 2.2 Study Procedure

Written informed consent was obtained from the parent/guardian, while written assent was obtained from the adolescent. At the first visit, the adolescent was screened for MRI eligibility as per procedures determined by the University of Oregon's Lewis Center for Neuroimaging, and they completed the vocabulary and matrix reasoning subtests of the Wechsler Abbreviated Scale of Intelligence (second edition; Wechsler, 1999). They were given a saliva kit with instructions on sample collection, which were then completed at home. At the second lab visit, typically scheduled around one month after their first visit, participants returned the saliva kit, provided a sample of their hair and completed the MRI scan and questionnaires on pubertal development. Participants were compensated for their time, and all materials and procedures were approved by the Institutional Review Board at the University of Oregon.

### 2.3 Pubertal assessment

*2.3.1 Tanner stage.* Participants completed the picture-based interview of puberty (PBIP). They viewed stylised line drawings corresponding to the five Tanner stages of breast and pubic hair development, and were asked to choose images that best reflected their own

development (Morris & Udry, 1980). We averaged responses relating to their current stage of development (and did not use responses relating to their next closest stage of development).

*2.3.2 Pubertal Development Scale.* Participants also completed the Pubertal Development Scale (PDS), which assesses height, growth, body hair and skin changes, as well as breast development and menarche in females. It consists of five questions that are scored on a 4-point scale ranging from "no physical changes" to "development seems complete", with the only exception being the yes/no item on menarche. The Shirtcliff et al. (2009) method was used to convert the PDS into a 5-point scale, in order to approximate Tanner staging. This method provides scores for two subscales (adrenal and gonadal development), as well as an overall puberty score.

*2.3.3 Pubertal composite score.* Three composite scores were created by combining the PBIP and PDS. The PBIP pubic hair and PDS adrenal scores were averaged to obtain a composite adrenal score, while the PBIP breast and PDS gonadal scores were averaged to obtain a composite gonadal score. Finally, the composite adrenal and gonadal scores were averaged to create an overall pubertal stage score. Correlations between the PDS, PBIP and composite scores are presented in Figure S1.

**2.4 Hormonal assessment**

*2.4.1 Saliva samples.* Participants provided an average of four saliva samples at home (range: 1-7 samples). Samples were provided on awakening, prior to the consumption of food or tooth brushing. Participants collected approximately 2 mL of saliva via passive drool, using a straw, into vials. Along with each saliva sample, participants completed a form that recorded the date and time of collection. They also noted if they had been sick in the prior 24 hours, or had taken any medications during the previous day. These variables were examined as

potential confounds during the calculation of basal saliva hormone concentrations (see section "2.6.1 Hormone confounds") for further detail.

After saliva collection, each vial was frozen in the family's freezer, within a provided cooler bag. All the samples were then transported in the cooler bag, with additional Techni-ice to ensure they remained frozen, to the University of Oregon at the second lab visit. Upon arrival at the University of Oregon, 5% of saliva kits (N=7) were fully thawed. Samples were stored in a -80°C freezer in Oregon until shipped (overnight with dry ice) to the Stress Physiology Investigative Team laboratory at the Iowa State University.

*2.4.2 Hair samples.* At the second lab visit, approximately 100 mg of hair was collected from participants, near the posterior vertex of the scalp. Participants also completed a brief survey about their hair, on potential covariates that have previously been considered to influence hormone concentrations in hair samples (Greff et al., 2018; Kristensen et al., 2017). This included whether they i) had curly hair, ii) had permed or colored their hair (along with time frames), and iii) felt their hair was relatively sweaty ("Do you feel like you sweat relatively frequently/heavily on the head?"). Hair samples were stored in foil pouches and batch-shipped overnight at room temperature to the Stress Physiology Investigative Team laboratory at Iowa State University.

*2.4.3 Hormone assays & processing.* For both hair and saliva samples, hormones were assayed using enzyme-immunoassay (ELISA) kits (www.salimetrics.com) for testosterone, DHEA and estradiol. For hair, samples were washed, ground, extracted, reconstituted and assayed using methodology described by Wang et al. (2019); estradiol required a double-extraction to purify the sample prior to assay. The 5cm closest to the scalp was assayed, and as hair grows approximately 1cm per month (range of 0.6–1.4 cm; Pragst & Balikova, 2006), the results are reflective of cumulative hormone levels from roughly the prior 5 months. Each hormone was assayed in duplicate. Samples were re-run when the optical density coefficient

of variation (CV) was greater than 7%, if enough sample was left over to do so. All hormones for each participant were assayed on the same day to minimize freeze-thaw cycles. Moreover, all saliva samples from each participant were assayed on the same plate, so as to minimize variation in hormone concentrations that may be attributable to plate differences (i.e., inter-assay CVs). Mean hormone concentrations and intra- and inter-assay CVs are presented in Table S2.

The number of mean salivary hormone concentrations (of the duplicates) that were non-detectable were as follows: *DHEA N:* participant=20, sample=38; *testosterone N:* participant=3, sample=3; *estradiol N:* participant=13, sample=23. Mean hormone concentrations that were non-detectable and left-censored were substituted using the following rules: *1)* If other samples from the participant (i.e., from other sampling days) were also not detectable, the mean of all samples were replaced with the lower limit of sensitivity (DHEA: 5 pg/ml; testosterone: 1 pg/ml; estradiol: 0.1 pg/ml). *2)* If other samples from the participant were detectable and 50% or more of the remaining samples were below the inter-quartile range (IQR) for the distribution, the mean of the non-detectable sample was replaced with the lower limit of sensitivity for that assay. *3)* If other samples from the participant were detectable and less than 50% of the remaining samples were below the IQR for the distribution, (i.e., most of the remaining samples were detectable and higher than the bottom quartile), the mean of the non-detectable sample was considered "missing". For mean salivary hormone concentrations that were right censored, the following rules applied: *1)* If 50% or more of the remaining samples were above the top IQR, the right censored sample was replaced with the upper limit of the standards for that assay (DHEA: 1000 pg/ml; Testosterone: 600 pg/ml; Estradiol: 32 pg/ml). *2)* If less than 50% of the remaining samples were above the top IQR (i.e., most of the samples were lower than the top quartile), the mean of the right censored sample was considered "missing".

Mean hair hormone concentrations (of the duplicates) that were non-detectable and right-censored were substituted with the upper limit of the standards for that assay (N: DHEA=0, testosterone=2, estradiol=0). There were no mean hair hormone concentrations that were non-detectable and left-censored.

Hair and saliva hormone concentrations were log-transformed in order to deal with positive skew and kurtosis prior to analysis. Finally, outliers greater than 3 standard deviations (Hair N: DHEA=1; testosterone=3, estradiol=1; Saliva N: DHEA=0, testosterone=7, estradiol=7) were winsorized to the values of +3 SD, plus increments of 0.01 pg/ml to maintain the ordinal value of the outliers. Refer to "2.6 Statistical analyses" for further information on extracting "basal" saliva hormone concentrations and the examination of potential hormonal confounds.

**2.5 MRI assessment**

Data was acquired on a 3T Siemens Skyra MRI scanner at the Lewis Center for Neuroimaging at the University of Oregon. A high-resolution T1-weighted structural image was collected with the MP-RAGE sequence (TE=3.41 ms, TR=2500 ms, flip angle=7°, 1.0 mm slice thickness, matrix size=256 x 256, FOV=256 mm, 176 slices, bandwidth=190 Hz/pixel). These images were subsequently processed on a high performance computing cluster at the University of Oregon. Cortical reconstruction was performed using the FreeSurfer (v 6.0) image analysis suite (http://surfer.nmr.mgh.harvard.edu/), which provides tools for reconstructing topologically and geometrically accurate surface models of the inner and outer cortical boundaries, thus deriving anatomical measures such as cortical thickness and volume. In addition, FreeSurfer includes tools for segmentation of deep grey matter structures, thus providing volumetric estimates of the subcortex.

The quality of the raw images was visually inspected and rated on a three-point scale (1= good, 2=okay, 3=bad) by trained researchers, focusing on the presence of Gibbs rings and blurring (or sharpness) of images. Each participants' data was examined by two separate raters, and inconsistencies in ratings were resolved by a senior researcher. Poorest quality images (i.e., rating of 3; N = 25) were excluded from analyses. In addition, ratings of 2 (N = 112) were re-examined by two senior researchers to determine inclusion. Of these, another 27 were deemed to have major motion-related issues and excluded from analyses, leaving a final sample size of 112 participants that had either no motion or minor motion-related issues that were limited to one area. In addition, the FreeSurfer processed images were visually examined (by a single researcher) to ensure quality of the cortical reconstruction (i.e., accuracy of grey-white and grey-pial boundaries). No further participants were excluded based on failures of cortical re-construction and no manual correction of processed images was undertaken. FreeSurfer's default segmentation was used to obtain volumetric estimates for 14 subcortical structures (7 per hemisphere), and the Desikan Killiney parcellation was extracted to obtain thickness estimates for 68 cortical structures (34 per hemisphere). Given that only one study has thus far examined associations between puberty and surface area of cortical structures (Herting et al., 2015), we focused on thickness estimates to be consistent with prior literature (thus allowing us to relate current findings to existing research).

### 2.6 Statistical analyses

*2.6.1 Hormone confounds.* In order to identify important covariates that should be accounted for within statistical models of brain-hormone associations, we first examined the relationship between hormone concentrations (from hair and saliva) and a number of potential extraneous variables. For saliva hormone concentrations, we examined 1) the effect of waking time and the time between waking and sample collection, 2) additional variance

explained by incorporating collection during a weekend (yes/no), 3) illness (yes/no), and 4) medication use (categorised on biochemical mechanisms of action). Waking time and time between waking and sample collection were significantly associated with testosterone and DHEA concentrations, respectively. The binary "weekend" variable did not explain any additional variance for any hormone. Neither illness nor medication use were associated with testosterone, DHEA or estradiol. Based on these results, waking time and time between waking and sample collection were controlled while extracting basal salivary hormone concentrations (see next section 2.6.2 for further detail).

For hair hormone concentrations, potential confounds included binary variables for 1) the effect of hair coloring within the last 6 months (yes/no), 2) hair perming within the last 6 months (yes/no), 3) curly hair (yes/no), and 4) sweatiness of hair using a three-point Likert scale (yes/maybe/no). Each of these four variables were examined individually. None were found to be associated with DHEA, testosterone or estradiol concentrations in hair samples ($p < 0.05$), respectively. As such, no confounds were included in statistical analyses of hair hormone concentrations.

*2.6.2 Basal saliva hormones.* Individual "basal" level saliva hormone concentrations across the various samples were identified with linear mixed models, using the *lmer* package in R (Bates et al., 2015). Specifically, the following model was run for each hormone: $Y_{ij} = \beta_0 + \beta_1 (\text{waking time})_{ij} + \beta_2 (\text{time between waking and collection})_{ij} + u_j + e_{ij}$. All models accounted for $i^{th}$ timepoint for each $j^{th}$ subject, with a random intercept ($u_j$) and residual error term ($e_{ij}$). The random effects from these models reflect "basal" hormone concentrations across the repeated measurements that accounts for within-individual variance. These random effects

were extracted and used in subsequent analyses. Basal hormone concentrations were only calculated for participants with two or more samples, given that any single sample may have been strongly influenced by state-level factors. Therefore, participants with only one sample were coded as having missing data, and imputation techniques were employed to deal with missingness (see next section 2.6.3).

*2.6.3 Missing data.* While the final sample size (N = 112) was comprised of participants with useable, good quality, structural MRI, a smaller subset of these individuals had missing independent variables. Seven participants were missing (composite) pubertal stage (i.e., PDS and PBIP), 4 were missing (basal) saliva hormones, 12 were missing hair DHEA, 14 were missing for hair testosterone, and 39 were missing hair estradiol concentrations. Multiple imputation was conducted using the *Mice* package in R (Buuren & Groothuis-Oudshoorn, 2011). Imputation was conducted across the entire TAG sample of 174, with the aim of improving model prediction by utilizing all available data. Variables included in the imputation model were age at each lab visit, (mean) hair hormone concentrations, basal saliva hormone concentrations, anthropometric data (i.e., height, weight, waist measurements), pubertal composite scores (for overall, adrenal and gonadal scales), and parent-reported PDS scores (for overall, adrenal and gonadal scales). Mean variables across the imputed dataset were calculated and used in subsequent statistical (neuroimaging) analyses.

*2.6.4 Neuroimaging analyses.* Analyses were conducted in R using subcortical volume and cortical thickness estimates extracted from FreeSurfer v6.0. Correlations were examined between these measures of brain structure and i) age, ii) pubertal stage, ii) adrenal physical changes, iii) gonadal physical changes, iv) hair hormone concentrations, and v) basal salivary

hormone concentrations. Across the two different hormone methodologies, correlations were conducted for testosterone, estradiol and DHEA separately. For hair and saliva hormone concentrations, we also characterized changes in correlations when controlling for pubertal stage. To do so, we calculated partial correlations between hormone-brain structure that accounted for pubertal stage, and plotted the difference between these partial correlations and Pearson's R (i.e., change in R when controlling for pubertal stage).

We also examined whether hair and saliva hormone concentrations explained additional variance while accounting for one another using log-likelihood ratio tests (specifically the *lmtest* package in R; Zeileis & Hothorn, 2002). To examine unique variance associated with hair testosterone, a log-likelihood ratio test compared a baseline model with salivary testosterone concentrations (i.e., cortical thickness predicted by salivary testosterone) and a model with hair testosterone concentrations incorporated (i.e., cortical thickness predicted by salivary testosterone and hair testosterone). Conversely, to examine unique variance associated with salivary testosterone, a log-likelihood ratio test compared a baseline model with hair testosterone concentrations (i.e., cortical thickness predicted by hair testosterone) and a model with salivary testosterone concentrations incorporated (i.e., cortical thickness predicted by hair testosterone and salivary testosterone). Similar models were run for DHEA and estradiol. We did not correct for multiple comparisons given the novelty of analyses utilizing hair hormone concentrations in neuroimaging research.

## 3. Results

The associations between age, puberty and hormone variables are presented in Figure 1.

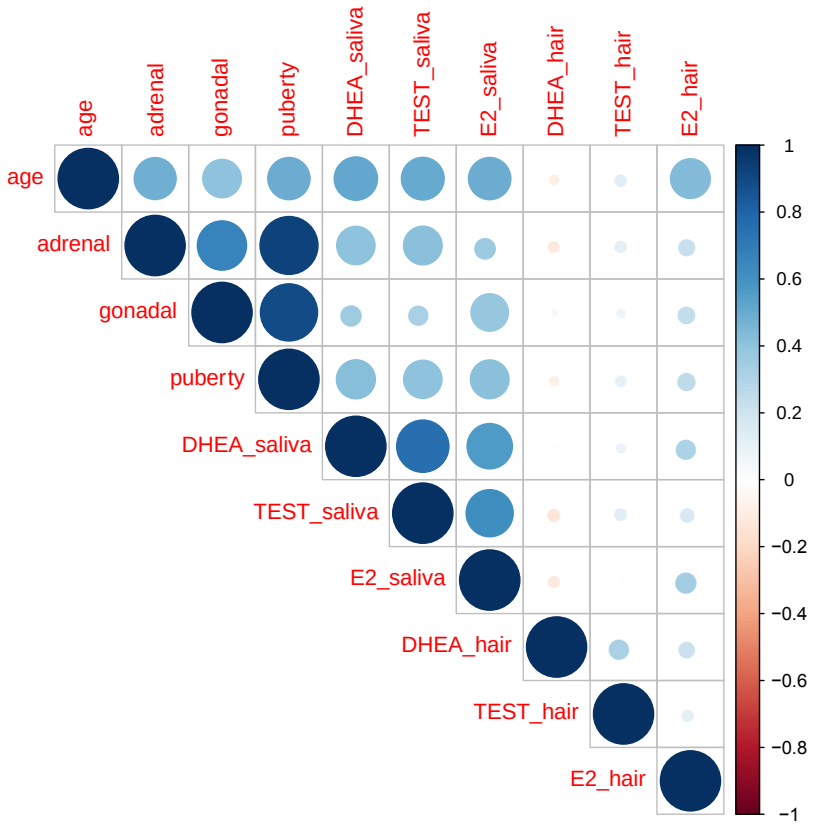


*Figure 1.* Correlation (Pearson's R) matrix of age, puberty, and hormones. TEST = testosterone, E2 = estradiol

### 3.1 Observed physical indices

Correlations between brain structure and i) age, and ii) composite pubertal scores are illustrated in Figure 2. Reductions in cortical thickness were identified across most of the brain with increasing age and pubertal stage. The strongest associations were evident in the bilateral medial and lateral surfaces of the occipital lobe (i.e., cuneus, precuneus and lateral occipital cortex), with Pearson's R extending up to 0.30 and unstandardized regression coefficients up to 0.05 (i.e., approximately 2% change per year or pubertal stage). Similar associations were also present in the frontal lobe with age, including reductions in bilateral

orbitofrontal and right cingulate thickness with age. Of the subcortical structures, the strongest association with age was identified in the right nucleus accumbens, which exhibited smaller volumes with increasing age. The strongest associations with pubertal stage were identified in the bilateral pallidum, which exhibited larger volumes with increasing pubertal stage (see Table 1). Refer to Table S3 for *p* values associated with correlation coefficients, and Table S4 for unstandardized coefficients.

When considering adrenal and gonadal scores, similar to the pubertal stage composite, negative associations were evident in the occipital and parieto-occipital cortices. Adrenal scores exhibited stronger associations with left lateral prefrontal thickness relative to gonadal changes. Both adrenal and gonadal scores were also positively associated with right pallidum volume, consistent with pubertal stage effects.

Table 1. *Hormonal associations with subcortical volume (Pearson's R reported)*

| | | | | | Testosterone | | | DHEA | | | Estradiol | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Region** | **Age** | **Puberty** | **Adrenal** | **Gonadal** | **Hair** | **Saliva** | **Difference** | **Hair** | **Saliva** | **Difference** | **Hair** | **Saliva** | **Difference** |
| Left Accumbens | -0.15 | -0.04 | -0.04 | -0.04 | -0.08 | -0.04 | -0.04 | -0.08 | -0.12 | 0.04 | -0.11 | -0.09 | -0.02 |
| Left Amygdala | 0.09 | 0.10 | 0.04 | 0.15 | 0.03 | 0.15 | -0.12 | -0.02 | 0.11 | -0.13 | 0.10 | 0.01 | 0.09 |
| Left Caudate | -0.08 | -0.07 | -0.06 | -0.06 | -0.11 | 0.01 | -0.12 | 0.00 | -0.01 | 0.01 | -0.04 | -0.02 | -0.02 |
| Left Hippocampus | 0.03 | 0.01 | -0.01 | 0.02 | -0.04 | 0.02 | -0.06 | -0.01 | 0.01 | -0.02 | 0.11 | -0.03 | 0.14 |
| Left Pallidum | -0.01 | 0.17 | 0.16 | 0.14 | -0.09 | 0.23 | -0.32 | -0.08 | 0.20 | -0.28 | 0.05 | 0.12 | -0.07 |
| Left Putamen | -0.09 | 0.04 | 0.02 | 0.06 | -0.06 | 0.06 | -0.12 | -0.08 | 0.12 | -0.20 | 0.03 | 0.10 | -0.07 |
| Left Thalamus | 0.08 | -0.03 | 0.01 | -0.06 | -0.09 | 0.14 | -0.23 | -0.04 | 0.10 | -0.14 | 0.07 | 0.11 | -0.04 |
| Right Accumbens | -0.20 | -0.11 | -0.15 | -0.05 | -0.03 | -0.02 | -0.01 | -0.06 | -0.05 | -0.01 | -0.10 | -0.10 | 0.00 |
| Right Amygdala | 0.05 | 0.06 | 0.00 | 0.11 | 0.03 | 0.10 | -0.07 | -0.02 | 0.10 | -0.12 | 0.05 | -0.07 | 0.12 |
| Right Caudate | 0.00 | 0.00 | 0.00 | 0.00 | -0.11 | 0.04 | -0.15 | 0.01 | 0.03 | -0.02 | 0.01 | 0.02 | -0.01 |
| Right Hippocampus | 0.01 | -0.05 | -0.03 | -0.07 | 0.02 | 0.03 | -0.01 | -0.01 | 0.04 | -0.05 | 0.12 | -0.08 | 0.20 |
| Right Pallidum | 0.06 | 0.18 | 0.17 | 0.16 | -0.09 | 0.25 | -0.34 | -0.12 | 0.21 | -0.33 | 0.02 | 0.14 | -0.12 |
| Right Putamen | -0.05 | 0.05 | 0.03 | 0.05 | -0.07 | 0.12 | -0.19 | -0.07 | 0.11 | -0.18 | 0.04 | 0.14 | -0.10 |
| Right Thalamus | 0.08 | -0.01 | 0.00 | -0.03 | -0.02 | 0.14 | -0.16 | -0.02 | 0.12 | -0.14 | 0.04 | 0.11 | -0.07 |

Note: Difference = Hair R – Saliva R. Negative "difference" values indicate stronger negative associations with hair, and positive values indicate stronger negative associations with saliva

### 3.2 Hormonal indices

*Cortex:* Correlations between brain structure and saliva and hair hormones are illustrated in Figure 3, and associated *p* values are reported in Table S3. Figure 4 illustrates regions where saliva or hair hormones explain unique variance in cortical thickness beyond one another, and associated model fit statistics are reported in Tables S5 and S6.

Hair DHEA concentrations were most strongly (negatively) correlated with thickness in the left ventrolateral PFC extending to bilateral insula, as well as left rostral ACC and right pericalcarine. Hair DHEA also explained unique variance in these regions over and above salivary DHEA. In comparison, salivary DHEA concentrations were most strongly (negatively) related to the right cuneus thickness and bilateral occipito-parietal cortices, explaining unique variance in these structures beyond hair concentrations.

Hair testosterone concentrations were more strongly (negatively) correlated with cingulate thickness than saliva concentrations, and explained unique variance in the left rostral ACC and bilateral isthmus cingulate cortex beyond saliva concentrations. Salivary testosterone concentrations were more strongly (negatively) correlated with right occipital and left parietal thickness than hair concentrations, and explained unique variance in these regions beyond hair concentrations. Salivary testosterone concentrations also exhibited strong positive correlations with thickness of the bilateral temporal cortices (i.e., transverse temporal and right superior temporal), and positive associations in the transverse temporal cortices explained unique variance in brain structure beyond hair concentrations.

Hair estradiol concentrations were more strongly negatively correlated with cortical thickness estimates than salivary estradiol across much of the brain. Moreover, hair estradiol concentrations explained additional variance in the bilateral prefrontal and temporal cortices above salivary estradiol. Salivary estradiol concentrations was positively correlated with thickness of the left medial orbitofrontal cortex, improving model fit in this region beyond hair concentrations.

Figure 5 highlights changes in the hormone-cortical thickness associations when controlling for pubertal stage. Specifically, it highlights the difference between the partial R (e.g., thickness = hair DHEA + pubertal stage) and the Pearson R (e.g., thickness = hair DHEA). There was minimal change in hair DHEA, testosterone and estradiol correlations with the cortical thickness when controlling for pubertal stage. For saliva hormone concentrations, the strongest effects were reductions in negative correlations between hormone levels and parieto-occipital cortices when accounting for pubertal stage.

*Subcortex:* When considering subcortical structures, salivary DHEA and testosterone concentrations were most strongly (positively) correlated with bilateral pallidum volumes (see Table 1). Salivary testosterone concentrations improved model fit of right pallidum beyond hair hormone concentrations ($\chi^2$ (1) = 4.795, p = 0.029), with a similar trending effect in the left pallidum ($\chi^2$ (1) = 3.699, p = 0.054). While estradiol concentrations were not as strongly related to any subcortical structures, it is interesting to note that strongest correlations were identified in the bilateral hippocampus. All other model fit statistics are presented in Tables S5 and S6. Controlling for pubertal stage resulted in minimal change in

the relationship between hormone concentrations and subcortical structures. Differences between partial and Pearson's correlations for the subcortex are reported in Table S7.

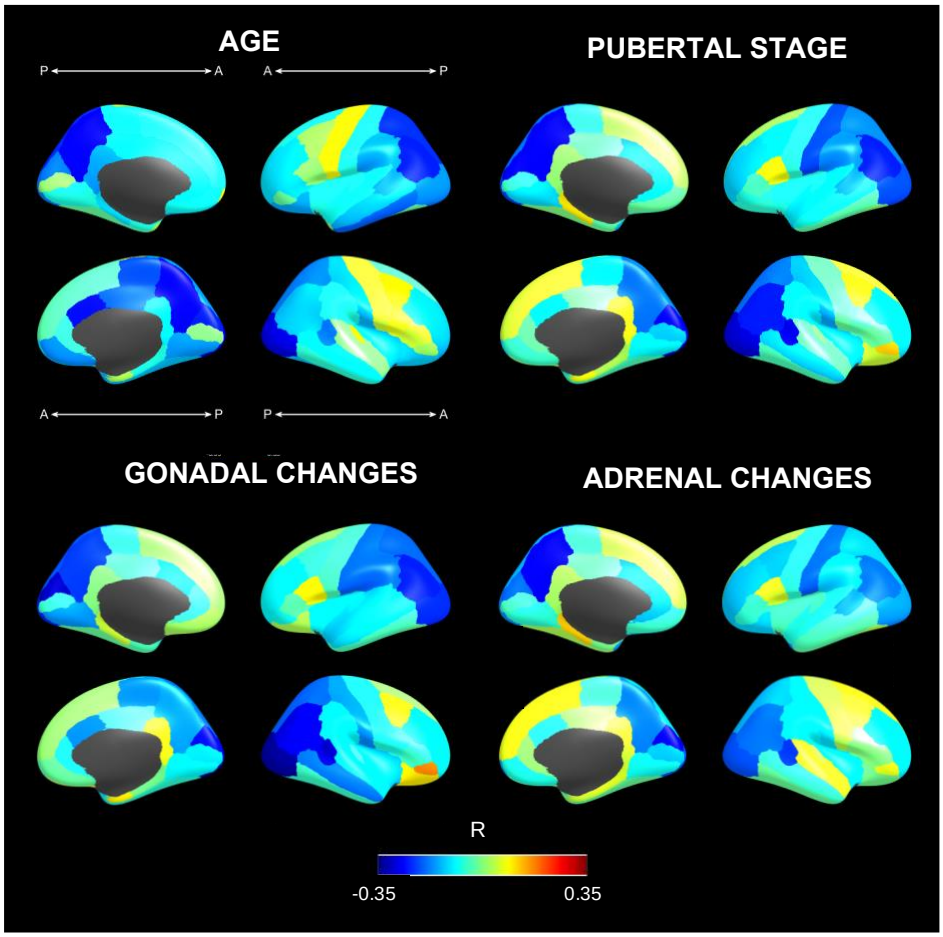


*Figure 2.* Age and pubertal associations with cortical thickness. Figure depicts Pearson's R.

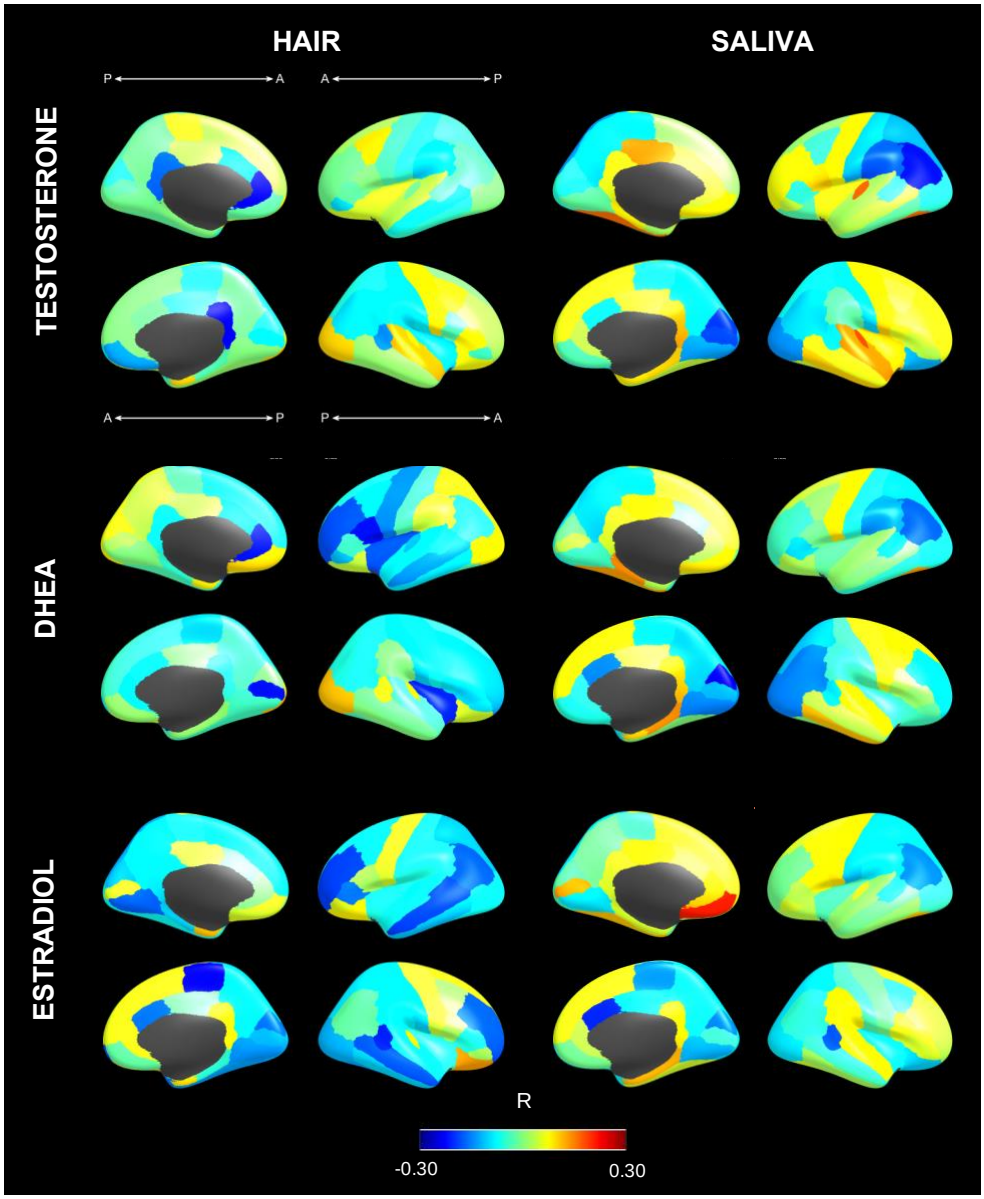


*Figure 3.* Hormonal associations with cortical thickness. Figures depict Pearson's R.

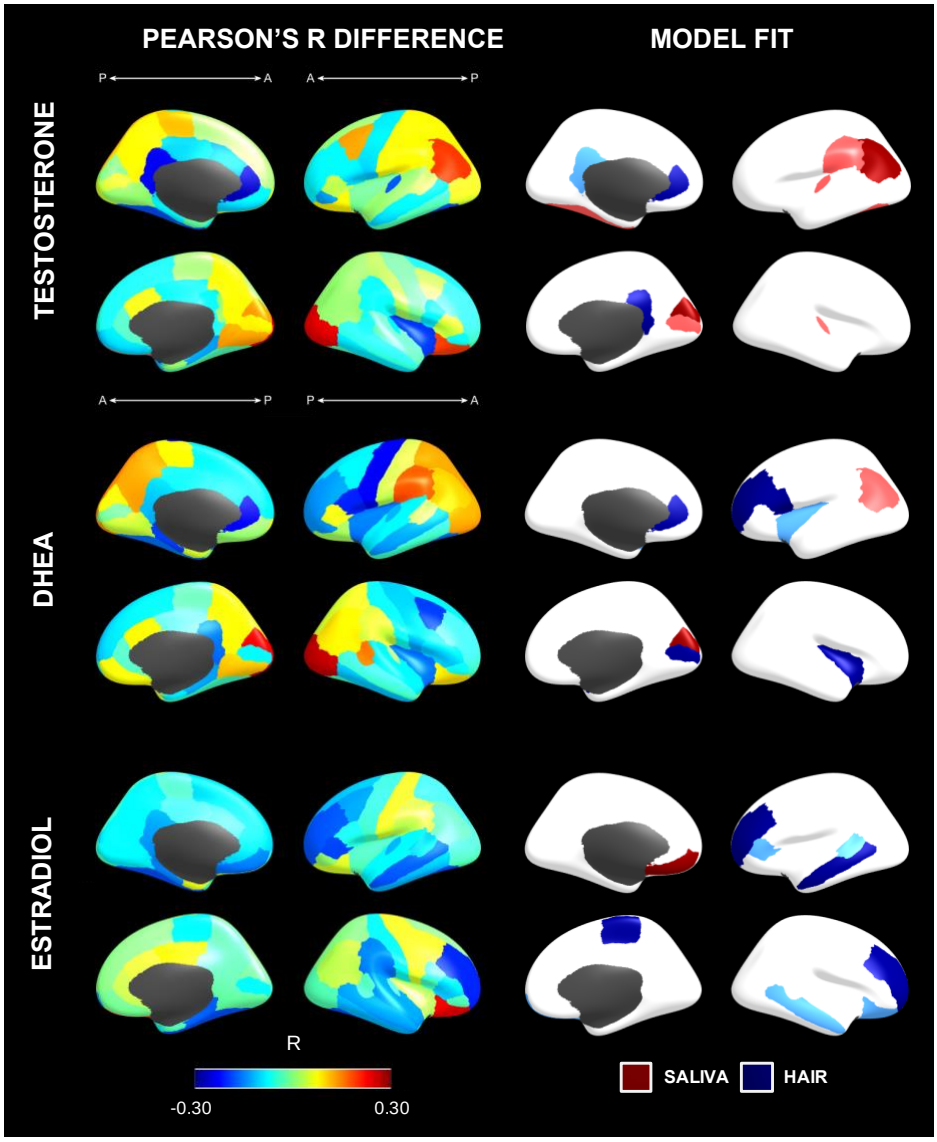


*Figure 4.* "Pearson's R difference" between saliva and hair hormone associations with cortical thickness (i.e. Saliva R – Hair R). Negative values (blue) indicate stronger negative associations with hair, and positive values (red) indicate stronger negative associations with saliva. "Model fit" illustrates regions where hair and saliva each explain unique variance beyond the other hormone method. Red and blue highlight improvements in model fit with saliva and hair hormones, respectively. Light colours represent trending effects ($p < 0.08$).

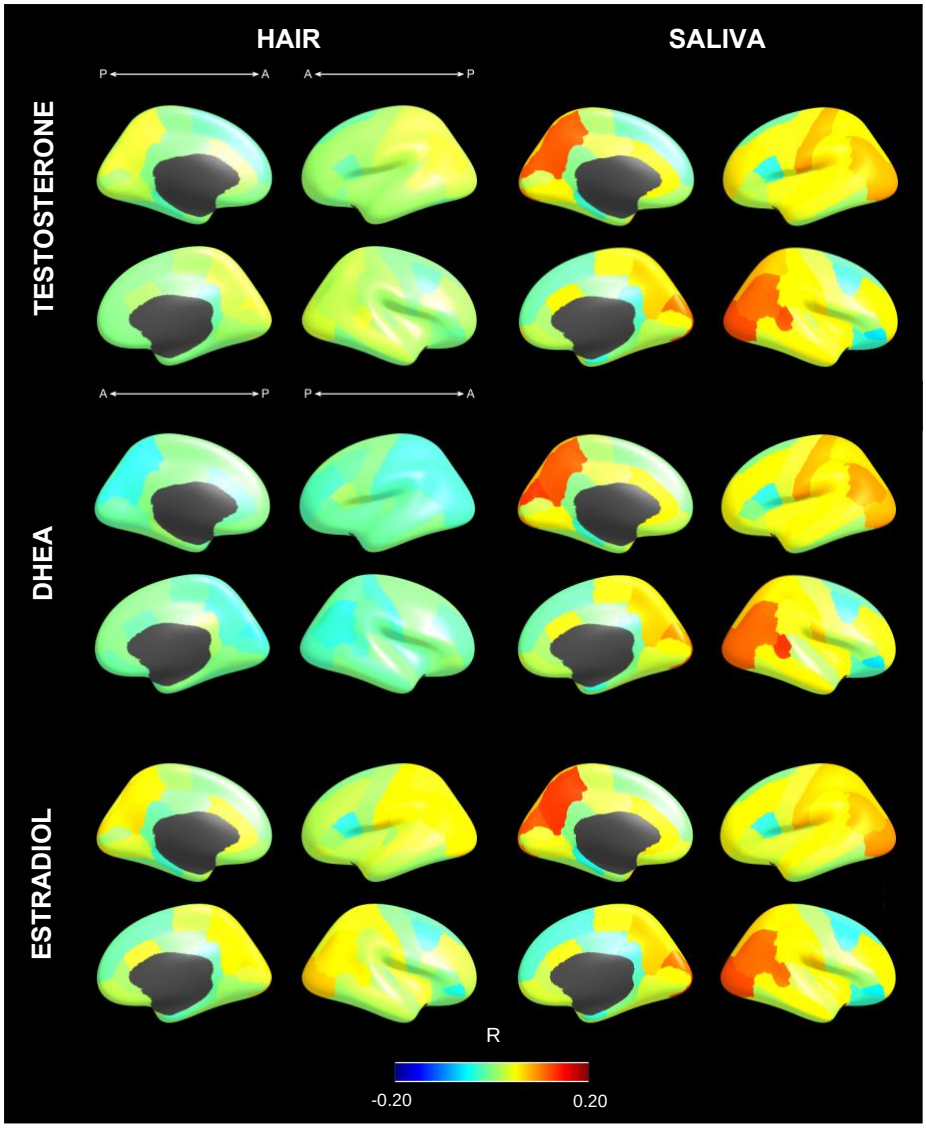


*Figure 5.* Change in hormonal associations with cortical thickness when controlling for pubertal stage. Figure depicts the difference between the partial R (controlling for pubertal stage) and the Pearson R. Negative values indicate more negative or less positive correlations (in blue) when controlling for pubertal stage, and positive values indicate less negative or more positive correlations (in red).

## 4. Discussion

The primary aim of this study was to examine the utility of incorporating hormonal assays of hair samples in pubertal neuroimaging research. To do so, we examined associations between

brain structure and multiple pubertal indices, including self-reported pubertal stage and hormone concentrations from saliva and hair samples. Consistent with the well-established phenomenon of cortical thinning during the second decade of life, our investigation revealed negative associations between pubertal stage and brain structure. Pubertal hormones in saliva samples exhibited overall weak associations with brain structure, and controlling for pubertal stage removed considerable variance in posterior (parieto-occipital) cortices. In comparison to saliva, hormone concentrations in hair exhibited stronger negative associations with cingulate and lateral prefrontal cortical thickness. Moreover, controlling for pubertal stage resulted in minimal change in the association between hair hormone concentrations and brain structure, suggesting that hair hormones may reflect unique developmental processes that are not indexed by self-report measures of physical pubertal characteristics.

Examination of age and pubertal stage revealed parallel associations with cortical thickness. The strongest effects for both indices of maturation were negative correlations in the posterior cortex, across much of the occipital and parietal lobes. These findings are somewhat consistent with prior longitudinal research that has identified posterior to anterior patterns of maturation (Tamnes et al., 2013). Despite our hypotheses that indices of adrenal and gonadal changes would provide unique information about brain structure beyond the global metric of pubertal stage, this was not the case for most of the brain. Rather, adrenal and gonadal changes exhibited very similar associations with brain structure to pubertal stage. There is preliminary evidence from prior research that each aspect of physical development (i.e., hair, breast, menarche) exhibits differential associations with brain structure. For example, negative associations between amygdala volume and breast development has been identified in females (Blanton, Cooney, Joormann, Eugène, et al., 2012; Hu, Pruessner, Coupé, & Collins, 2013), and positive associations with hair and skin changes has been found in males (Hu et al., 2013). A recent study of anatomical white-matter

connectivity also found unique effects of adrenal and gonadal physical changes, with adrenal changes relating to increased white matter integrity in the thalamus and precentral gyrus, but gonadal changes relating to reductions in white matter integrity in the corpus callosum, superior and anterior corona radiata, and superior frontal gyrus (Herting et al., 2017). Our lack of findings may in part be attributable to the relatively young age range of our sample (10-13 years), as an even distribution of the latter stages of gonadal changes were not yet apparent. Further research is thus warranted to examine this hypothesis in a sample with a wider age range that can capture the range of physical changes associated with adrenarche and gonadarche, or ideally in a longitudinal sample that can examine intra-individual developmental processes.

When considering hormones, we identified strong negative associations (of medium effect sizes) between hair estradiol concentrations and cortical thickness in the lateral prefrontal and temporal cortices. Comparatively, salivary estradiol concentrations exhibited weaker associations with cortical thickness, with the strongest effect being positive correlations in the left medial orbitofrontal cortex. Prior studies have predominantly found negative associations between estradiol and cortical thickness in adolescents, although the region of associations has varied considerably (Brouwer et al., 2015; Koolschijn, Peper, & Crone, 2014a; Neufang et al., 2009; Peper et al., 2009). In the context of prior literature, our findings suggest that hair estradiol concentrations may be a valuable index of developmental processes. This period is particularly characterized by irregular fluctuations in gonadal hormones (Shirtcliff et al., 2009), and it is possible that even "basal" saliva hormone concentrations may be more confounded by these variations relative to hair samples, which are able to capture hormonal exposure over a much longer time frame. Nevertheless, given that salivary estradiol concentrations are more strongly correlated with self-reported pubertal stage, further research is needed to understand potential mechanisms.

We also identified negative associations between adrenal hormone concentrations and cortical thickness. Hair testosterone concentrations were most strongly negatively associated with cingulate thickness, while salivary concentrations were most strongly negatively associated with thickness of the left parietal and right occipital cortices. As with estradiol, prior research has most consistently identified negative associations. Some of these findings cluster in the frontal lobe (e.g., ACC and OFC; Koolschijn et al., 2014; Peper et al., 2009), while others cluster in the parietal and temporal cortices (Bramen et al., 2012; Neufang et al., 2009). Based on these varied findings, it appears that hair and saliva hormones may each provide unique information about testosterone-related cortical structure.

DHEA concentrations in hair, as with estradiol, exhibited stronger negative associations with cortical thickness in the PFC (i.e., left ventrolateral PFC, left rostral ACC) and insula. Comparatively, salivary DHEA, as with testosterone, exhibited slightly weaker (negative) associations that clustered in the parieto-occipital thickness. Only two prior studies have examined DHEA concentrations in relation in grey matter structure, with one focusing on pituitary volume and identifying positive associations in 9 year olds (Murray et al., 2016). The other study identified a reduction in positive associations between salivary hormone concentrations and the left dlPFC, right entorhinal and temporoparietal cortices with age (between 4-22 year olds), such that associations disappeared by 13 years of age (Nguyen et al., 2013). Our findings do not directly track with these (limited) prior findings, but highlight the need for further research to incorporate DHEA into pubertal investigations, particularly during early adolescence when both adrenal and gonadal development are likely to exert neurodevelopmental effects. Interestingly, both adrenal hormones (DHEA and testosterone) in saliva exhibit stronger negative associations in parieto-occipital cortices, similar to the effects identified for physical pubertal development.

Indeed, further insight into differences between hair and saliva hormone concentrations were gathered by covarying for pubertal stage. Controlling for pubertal stage reduced the strength of negative associations between salivary hormones and thickness across much of the parieto-occipital cortex, suggesting that salivary hormones were tracking with physical changes captured by self-reported puberty or Tanner staging. Comparatively, associations between hair hormone concentrations and cortical thickness were minimally overlapping with pubertal stage, suggesting that other processes not captured by self-report pubertal stage may be responsible. Of relevance to this hypothesis, hair DHEA concentrations were recently shown to predict physical development (specifically height) in a sample of children showing minimal secondary sexual characteristics (Smith et al., in press). Thus one possibility is that hair hormone concentrations may be a sensitive marker of biological changes occurring during the early stages of puberty. Future investigations of associations between hair hormones concentrations and brain structure in pre-pubertal children may further inform this hypothesis.

Differences between hair and saliva may also reflect variations in the time frame indexed by these methods. A recent investigation of DHEA and testosterone concentrations in young female adults revealed strong and significant associations between hair and saliva samples indexing a 2 month period, but the authors did not examine stability of hair-saliva associations across different time frames (Wang et al., 2019). The authors did find, however, that the stability of hair androgen concentrations decreased over time. Specifically, hormone concentrations from 1cm hair segments were most strongly correlated to the neighbouring 1cm segment, and the strength of this association decreased with distance between hair segments. Consequently, hormone concentrations averaged across 5cm hair segments in this study (thus reflecting prior 5 months of hormonal exposure) may not be strongly correlated with basal saliva concentrations from a 4 week period. Moreover, associations across

different time frames may be even weaker during this period of peak pubertal development. Future research comparing saliva and hair hormone concentrations in a similar time frame within this age range will help us gain a better understanding of how these methods relate to one another during puberty.

It is also possible that chronic stress may play a role, as the limited studies of hair DHEA and testosterone have identified associations with (chronic) mental and physical stress, as well as psychopathology (Dettenborn et al., 2016; Ullmann et al., 2016). This includes one study of adolescents, which found stronger associations between externalizing symptoms and hair testosterone concentrations relative to salivary testosterone (with similar differences in effect sizes between hair and saliva to our findings; Grotzinger et al., 2018). However, Smith and colleagues (in press) found that hair concentrations of the primary stress hormone, cortisol, were only weakly associated with hair DHEA and testosterone concentrations in 8 year olds. As such, this explanation remains tentative and further research incorporating measures of stress are needed.

Findings should also be considered in light of other limitations. It is not possible to comment on developmental trajectories with this cross-sectional sample. While we have tried to identify consistencies with prior longitudinal research to support our conclusions, intra-individual analyses are needed to corroborate our findings. We also did not examine non-linear associations between puberty and brain structure as longitudinal research is most suited to examine such developmental trends. However, this may explain the lack of strong associations in the subcortex. Next, we chose not to incorporate age in our analyses of pubertal stage and hormones. When age is incorporated into statistical models of puberty, the resultant pubertal metric is more akin to "stage-for-age" (i.e., timing), where higher scores indicate greater maturation than same-aged peers. While both pubertal stage and timing are important, we are interested in normative progression through puberty as an alternate index

of development to age (as opposed to stage of development relative to peers). We also predominantly focused on correlation coefficients in this manuscript. We felt that a comprehensive characterization of effects sizes was warranted given that varied analytic strategies employed in prior research makes it difficult to identify consistent patterns of associations. A reliance on significance tests would also eliminate strong sub-threshold effects and bias the literature. However, we note that the size of our strongest effects (~ 0.30) is consistent with prior research that has reported "significant" correlation coefficients (Blanton et al., 2012; Nguyen et al., 2013; Peper et al., 2010).

In conclusion, our comprehensive characterization of pubertal associations with brain structure revealed extensive negative associations with pubertal development. Pubertal hormones in saliva samples exhibited strongest associations with brain structure in the occipito-parietal cortices and controlling for pubertal stage removed much of these associations. In comparison, hair hormone concentrations exhibited strongest negative associations with cingulate and lateral prefrontal cortical thickness. Moreover, controlling for pubertal stage resulted in minimal change in hair hormone concentrations, suggesting that developmental processes not captured by self-reported pubertal stage may be implicated.

## 5. Funding

This work was supported by the National Institute of Health grant R01 MH107418 (PI: Pfeifer).

## 6. References

1. Apter, D., & Vihko, R. (1985). Premenarcheal endocrine changes in relation to age at menarche. *Clinical Endocrinology*, *22*(6), 753–760.

2. Apter, D, Viinikka, L., & Vihko, R. (1978). Hormonal Pattern of Adolescent Menstrual Cycles. *The Journal of Clinical Endocrinology & Metabolism*, *47*(5), 944–954. https://doi.org/10.1210/jcem-47-5-944
3. Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67*(1), 1-48.<doi:10.18637/jss.v067.i01>.
4. Berenbaum, S. A., Beltz, A. M., & Corley, R. (2015). The importance of puberty for adolescent development: conceptualization and measurement. *Advances in Child Development and Behavior*, *48*, 53–92. https://doi.org/10.1016/bs.acdb.2014.11.002
5. Blanton, R. E., Cooney, R. E., Joormann, J., Eugène, F., Glover, G. H., & Gotlib, I. H. (2012). Pubertal stage and brain anatomy in girls. *Neuroscience*, *217*, 105–112. https://doi.org/10.1016/j.neuroscience.2012.04.059
6. Bramen, J. E., Hranilovich, J. A., Dahl, R. E., Chen, J., Rosso, C., Forbes, E. E., … Sowell, E. R. (2012). Sex matters during adolescence: testosterone-related cortical thickness maturation differs between boys and girls. *PLoS ONE*, *7*(3), e33850.
7. Bramen, J. E., Hranilovich, J. A., Dahl, R. E., Forbes, E. E., Chen, J., Toga, A. W., … Sowell, E. R. (2011). Puberty Influences Medial Temporal Lobe and Cortical Gray Matter Maturation Differently in Boys Than Girls Matched for Sexual Maturity. *Cerebral Cortex*, *21*(3), 636–646. https://doi.org/10.1093/cercor/bhq137
8. Brouwer, R. M., Koenis, M. M. G., Schnack, H. G., van Baal, G. C., van Soelen, I. L. C., Boomsma, D. I., & Hulshoff Pol, H. E. (2015). Longitudinal development of hormone levels and grey matter density in 9 and 12-year-old twins. *Behavior Genetics*, *45*(3), 313–323.

9. Buuren, S. van, & Groothuis-Oudshoorn, K. (2011). mice: Multivariate Imputation by Chained Equations in R. *Journal of Statistical Software*, *45*(1), 1–67. https://doi.org/10.18637/jss.v045.i03
10. Dettenborn, L., Kirschbaum, C., Gao, W., Spitzer, C., Roepke, S., Otte, C., & Wingenfeld, K. (2016). Increased hair testosterone but unaltered hair cortisol in female patients with borderline personality disorder. *Psychoneuroendocrinology*, *71*, 176–179. https://doi.org/10.1016/j.psyneuen.2016.05.026
11. Di Luigi, L., Baldari, C., Gallotta, M. C., Perroni, F., Romanelli, F., Lenzi, A., & Guidetti, L. (2006). Salivary steroids at rest and after a training load in young male athletes: relationship with chronological age and pubertal development. *International Journal of Sports Medicine*, *27*(9), 709–717. https://doi.org/10.1055/s-2005-872931
12. Eatough, E. M., Shirtcliff, E. A., Hanson, J. L., & Pollak, S. D. (2009). Hormonal reactivity to MRI scanning in adolescents. *Psychoneuroendocrinology*, *34*(8), 1242–1246.
13. Goddings, A.-L., Beltz, A., Peper, J. S., Crone, E. A., & Braams, B. R. (2019). Understanding the Role of Puberty in Structural and Functional Development of the Adolescent Brain. *Journal of Research on Adolescence: The Official Journal of the Society for Research on Adolescence*, *29*(1), 32–53. https://doi.org/10.1111/jora.12408
14. Greff, M. J., Levine, J. M., Abuzgaia, A. M., Elzagallaai, A. A., Rieder, M. J., & van Uum, S. H. (2018). Hair cortisol analysis: An update on methodological considerations and clinical applications. *Clinical biochemistry*.
15. Grotzinger, A. D., Mann, F. D., Patterson, M. W., Tackett, J. L., Tucker-Drob, E. M., & Harden, K. P. (2018). Hair and Salivary Testosterone, Hair Cortisol, and Externalizing Behaviors in Adolescents. *Psychological Science*, 956797617742981. https://doi.org/10.1177/0956797617742981

16. Havelock, J. C., Auchus, R. J., & Rainey, W. E. (2004). The rise in adrenal androgen biosynthesis: adrenarche. *Seminars in Reproductive Medicine*, *22*(4), 337–347. https://doi.org/10.1055/s-2004-861550
17. Herting, M. M., Gautam, P., Spielberg, J. M., Dahl, R. E., & Sowell, E. R. (2015). A Longitudinal Study: Changes in Cortical Thickness and Surface Area during Pubertal Maturation. *PLOS ONE*, *10*(3), e0119774. https://doi.org/10.1371/journal.pone.0119774
18. Herting, M. M., Kim, R., Uban, K. A., Kan, E., Binley, A., & Sowell, E. R. (2017). Longitudinal changes in pubertal maturation and white matter microstructure. *Psychoneuroendocrinology*, *81*, 70–79. https://doi.org/10.1016/j.psyneuen.2017.03.017
19. Herting, M. M., Gautam, P., Spielberg, J. M., Kan, E., Dahl, R. E., & Sowell, E. R. (2014). The role of testosterone and estradiol in brain volume changes across adolescence: a longitudinal structural MRI study. *Human Brain Mapping*, *35*(11), 5633–5645. https://doi.org/10.1002/hbm.22575
20. Hu, S., Pruessner, J. C., Coupé, P., & Collins, D. L. (2013). Volumetric analysis of medial temporal lobe structures in brain development from childhood to adolescence. *NeuroImage*, *74*, 276–287.
21. Jones, S. A., Morales, A. M., Lavine, J. B., & Nagel, B. J. (2017). Convergent neurobiological predictors of emergent psychopathology during adolescence. *Birth Defects Research*, *109*(20), 1613–1622. https://doi.org/10.1002/bdr2.1176
22. Juraska, J. M., & Willing, J. (2017). Pubertal onset as a critical transition for neural development and cognition. *Brain Research*, *1654*(Pt B), 87–94. doi:10.1016/j.brainres.2016.04.012
23. Kristensen, S. K., Larsen, S. C., Olsen, N. J., Fahrenkrug, J., & Heitmann, B. L. (2017). Hair dyeing, hair washing and hair cortisol concentrations among women from the healthy start study. *Psychoneuroendocrinology*, *77*, 182-185.

24. Koolschijn, P. C. M. P., Peper, J. S., & Crone, E. A. (2014). The Influence of Sex Steroids on Structural Brain Maturation in Adolescence. *PLOS ONE*, *9*(1), e83929. https://doi.org/10.1371/journal.pone.0083929

25. Marceau, K., Dorn, L. D., & Susman, E. J. (2012). Stress and puberty-related hormone reactivity, negative emotionality, and parent–adolescent relationships. *Psychoneuroendocrinology*, *37*(8), 1286–1298. https://doi.org/10.1016/j.psyneuen.2012.01.001

26. Marceau, K., Shirtcliff, E. A., Hastings, P., Klimes-Dougan, B., Zahn-Waxler, C., Dorn, L., & Susman, E. J. (2014). Within-adolescent coupled changes in cortisol with DHEA and testosterone in response to three stressors during adolescence. *Psychoneuroendocrinology*, *41*, 33–45. https://doi.org/10.1016/j.psyneuen.2013.12.002

27. Matchock, R. L., Dorn, L. D., & Susman, E. J. (2007). Diurnal and seasonal cortisol, testosterone, and DHEA rhythms in boys and girls during puberty. *Chronobiology International*, *24*(5), 969–990. https://doi.org/10.1080/07420520701649471

28. Morris, N. M., & Udry, J. R. (1980). Validation of a self-administered instrument to assess stage of adolescent development. *Journal of Youth and Adolescence*, *9*(3), 271–280. https://doi.org/10.1007/BF02088471

29. Murray, C. R., Simmons, J. G., Allen, N. B., Byrne, M. L., Mundy, L. K., Seal, M. L., … Whittle, S. (2016). Associations between dehydroepiandrosterone (DHEA) levels, pituitary volume, and social anxiety in children. *Psychoneuroendocrinology*, *64*, 31–39. https://doi.org/10.1016/j.psyneuen.2015.11.004

30. Neufang, S., Specht, K., Hausmann, M., Güntürkün, O., Herpertz-Dahlmann, B., Fink, G. R., & Konrad, K. (2009). Sex differences and the impact of steroid hormones on the developing human brain. *Cerebral Cortex (New York, N.Y. : 1991)*, *19*(2), 464–473.

31. Nguyen, T.-V., McCracken, J. T., Ducharme, S., Cropp, B. F., Botteron, K. N., Evans, A. C., & Karama, S. (2013). Interactive Effects of Dehydroepiandrosterone and Testosterone on Cortical Thickness during Early Brain Development. *Journal of Neuroscience*, *33*(26), 10840–10848. https://doi.org/10.1523/JNEUROSCI.5747-12.2013

32. Palmert, M. R., Hayden, D. L., Mansfield, M. J., Crigler, J. F., Crowley, W. F., Chandler, D. W., & Boepple, P. A. (2001). The longitudinal study of adrenal maturation during gonadal suppression: evidence that adrenarche is a gradual process. *The Journal of Clinical Endocrinology and Metabolism*, *86*(9), 4536–4542. https://doi.org/10.1210/jcem.86.9.7863

33. Peper, J. S., Brouwer, R. M., Schnack, H. G., van Baal, G. C., van Leeuwen, M., van den Berg, S. M., … Hulshoff Pol, H. E. (2009). Sex steroids and brain structure in pubertal boys and girls. *Psychoneuroendocrinology*, *34*(3), 332–342.

34. Peper, J. S., Brouwer, R. M., van Leeuwen, M., Schnack, H. G., Boomsma, D. I., Kahn, R. S., & Hulshoff Pol, H. E. (2010). HPG-axis hormones during puberty: a study on the association with hypothalamic and pituitary volumes. *Psychoneuroendocrinology*, *35*(1), 133–140.

35. Pragst, F., & Balikova, M. A. (2006). State of the art in hair analysis for detection of drug and alcohol abuse. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *370*(1–2), 17–49. https://doi.org/10.1016/j.cca.2006.02.019

36. Schulz, K. M., Molenda-Figueira, H. A., & Sisk, C. L. (2009). Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. *Hormones and Behavior*, *55*(5), 597–604. https://doi.org/10.1016/j.yhbeh.2009.03.010

37. Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: correspondence between hormonal and physical development. *Child Development*, *80*(2), 327–337. https://doi.org/10.1111/j.1467-8624.2009.01263.x

38. Sisk, C. L. (2017). Development: Pubertal Hormones Meet the Adolescent Brain. *Current Biology*, *27*(14), R706–R708. https://doi.org/10.1016/j.cub.2017.05.092

39. Smith, J., Johnson, K., Whittle, S., Allen, N. & Simmons, J. (in press). Measurement of cortisol, DHEA and testosterone in the hair of children: preliminary results and promising indications. *Developmental Psychobiology.*

40. Soliman, A., De Sanctis, V., & Elalaily, R. (2014). Nutrition and pubertal development. *Indian Journal of Endocrinology and Metabolism*, *18*(Suppl 1), S39–S47. https://doi.org/10.4103/2230-8210.145073

41. Stalder, T., & Kirschbaum, C. (2012). Analysis of cortisol in hair--state of the art and future directions. *Brain, Behavior, and Immunity*, *26*(7), 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002

42. Tamnes, C. K., Walhovd, K. B., Dale, A. M., Østby, Y., Grydeland, H., Richardson, G., … Fjell, A. M. (2013). Brain development and aging: Overlapping and unique patterns of change. *NeuroImage*, *68*, 63–74. https://doi.org/10.1016/j.neuroimage.2012.11.039

43. Tanner, J. M. (1962). *Growth at Adolescence (2nd edition)*. Oxford: Blackwell Publishing.

44. Ullmann, E., Barthel, A., Petrowski, K., Stalder, T., Kirschbaum, C., & Bornstein, S. R. (2016). Pilot study of adrenal steroid hormones in hair as an indicator of chronic mental and physical stress. *Scientific Reports*, *6*, 25842. https://doi.org/10.1038/srep25842

45. van Buuren, S., & Groothuis-Oudshoorn, K. (2011). mice: Multivariate Imputation by Chained Equations in R. *Journal of Statistical Software, 45*(3), 1-67. URL https://www.jstatsoft.org/v45/i03/

46. Vijayakumar, N., Op de Macks, Z., Shirtcliff, E. A., & Pfeifer, J. H. (2018). Puberty and the human brain: Insights into adolescent development. *Neuroscience and Biobehavioral Reviews*, *92*, 417–436. https://doi.org/10.1016/j.neubiorev.2018.06.004

47. Wang, W., Moody, S. N., Kiesner, J., Appiani, A. T., Robertson, O. C., & Shirtcliff, E. A. (2019). Assay validation of hair androgens across the menstrual cycle. *Psychoneuroendocrinology, 101*, 175-181.

48. Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence*. San Antonio, TX: The Psychological Corporation.

49. Wong, A. P.-Y., Pipitone, J., Park, M. T. M., Dickie, E. W., Leonard, G., Perron, M., … Paus, T. (2014). Estimating volumes of the pituitary gland from T1-weighted magnetic-resonance images: Effects of age, puberty, testosterone, and estradiol. *NeuroImage, 94*, 216–221. https://doi.org/10.1016/j.neuroimage.2014.02.030

50. Zeileis, A., & Hothorn, T. (2002). Diagnostic Checking in Regression Relationships. *R News 2*(3), 7-10. URL https://CRAN.R-project.org/doc/Rnews/

**Supplementary Material**

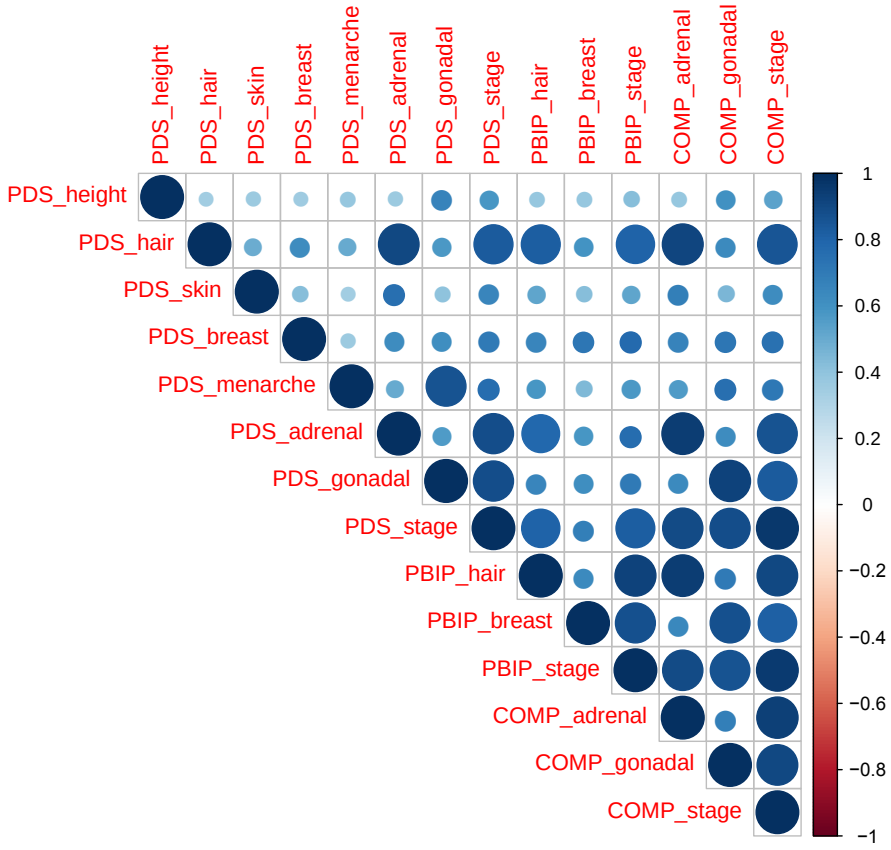


*Figure S1*. Correlations between the puberty composite (COMP) scores. PDS = Pubertal Development Scale. PBIP = Picture Based Interview of Puberty.

Table S1. *Race & ethnicity*

| | **N** | **%** |
|---|---|---|
| *Race* | | |
| Caucasian | 135 | 77.6 |
| American Indian/ Alaskan Native | 1 | 0.6 |
| Asian | 2 | 1.1 |
| Native Hawaiian/ Pacific Islander | 0 | 0 |
| African American | 2 | 1.1 |
| Multiracial | 34 | 19.5 |
| *Hispanic Ethnicity* | 28 | 16.1 |

Table S2. *Hormone concentrations and Intra- and inter-assay coefficients of variation*

| | **Concentrations** | | **Intra-assay CV** | | **Inter-assay CV** |
|---|---|---|---|---|---|
| **Hormone** | **Mean** | **SD** | **Mean** | **SD** | |
| *Saliva* | | | | | |
| DHEA (pgmg) | 104 | 127 | 10.48 | 16.66 | 29.3 |
| TEST (pgmg) | 40.4 | 24.1 | 1.80 | 1.94 | 22.9 |
| E2 (pgug) | 0.91 | 0.55 | 7.76 | 11.47 | 16.8 |
| *Hair* | | | | | |
| DHEA (pgmg) | 15.24 | 14.84 | 11.09 | 13.78 | 37.4 |
| TEST (pgmg) | 1.81 | 2.88 | 5.88 | 5.87 | 15.6 |
| E2 (pgug) | 37.80 | 15.82 | 5.02 | 5.54 | 10.7 |

TEST = testosterone, E2 = estradiol

Table S3. *Significance of Pearson's correlations (p values)*

| Hemi | Region | Age | Puberty | Adrenal | Gonadal | DHEA_hair | DHEA_saliva | E2_hair | E2_saliva | TEST_hair | TEST_saliva |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cortex* | | | | | | | | | | | |
| Left | Banks Superior Temporal Sulcus | 0.071 | 0.074 | 0.073 | 0.163 | 0.479 | 0.331 | 0.042 | 0.531 | 0.841 | 0.651 |
| Left | Caudal Anterior Cingulate | 0.258 | 0.075 | 0.068 | 0.137 | 0.266 | 0.929 | 0.898 | 0.657 | 0.231 | 0.669 |
| Left | Caudal Middle Frontal | 0.839 | 0.266 | 0.155 | 0.619 | 0.416 | 0.949 | 0.446 | 0.447 | 0.305 | 0.675 |
| Left | Cuneus | 0.050 | 0.003 | 0.038 | 0.001 | 0.552 | 0.910 | 0.204 | 0.810 | 0.594 | 0.582 |
| Left | Entorhinal | 0.448 | 0.782 | 0.951 | 0.690 | 0.404 | 0.757 | 0.259 | 0.634 | 0.978 | 0.842 |
| Left | Frontal Pole | 0.295 | 0.914 | 0.766 | 0.936 | 0.528 | 0.919 | 0.932 | 0.510 | 0.369 | 0.917 |
| Left | Fusiform | 0.856 | 0.964 | 0.726 | 0.800 | 0.758 | 0.172 | 0.495 | 0.259 | 0.794 | 0.085 |
| Left | Inferior Parietal | 0.007 | 0.008 | 0.034 | 0.008 | 0.622 | 0.059 | 0.056 | 0.084 | 0.873 | 0.020 |
| Left | Inferior Temporal | 0.034 | 0.725 | 0.790 | 0.668 | 0.360 | 0.607 | 0.267 | 0.955 | 0.623 | 0.841 |
| Left | Insula | 0.441 | 0.455 | 0.680 | 0.334 | 0.055 | 0.868 | 0.559 | 0.726 | 0.597 | 0.531 |
| Left | Isthmus Cingulate | 0.085 | 0.899 | 0.841 | 0.961 | 0.437 | 0.567 | 0.351 | 0.596 | 0.073 | 0.510 |
| Left | Lateral Occipital | 0.054 | 0.021 | 0.078 | 0.012 | 0.429 | 0.520 | 0.472 | 0.687 | 0.996 | 0.596 |
| Left | Lateral Orbitofrontal | 0.118 | 0.563 | 0.211 | 0.736 | 0.693 | 0.821 | 0.408 | 0.760 | 0.616 | 0.722 |
| Left | Lingual | 0.496 | 0.228 | 0.508 | 0.098 | 0.996 | 0.675 | 0.077 | 0.351 | 0.954 | 0.864 |
| Left | Medial Orbitofrontal | 0.414 | 0.850 | 0.585 | 0.835 | 0.291 | 0.328 | 0.584 | 0.028 | 0.831 | 0.423 |
| Left | Middle Temporal | 0.150 | 0.394 | 0.701 | 0.242 | 0.135 | 0.981 | 0.049 | 0.896 | 0.489 | 0.727 |
| Left | Paracentral | 0.417 | 0.620 | 0.697 | 0.611 | 0.870 | 0.286 | 0.516 | 0.734 | 0.628 | 0.384 |
| Left | Parahippocampal | 0.231 | 0.285 | 0.193 | 0.586 | 0.631 | 0.132 | 0.279 | 0.581 | 0.964 | 0.387 |
| Left | Pars Opercularis | 0.913 | 0.410 | 0.528 | 0.363 | 0.022 | 0.953 | 0.873 | 0.398 | 0.998 | 0.296 |
| Left | Pars Orbitalis | 0.859 | 0.434 | 0.552 | 0.410 | 0.254 | 0.739 | 0.498 | 0.611 | 0.338 | 0.708 |
| Left | Pars Triangularis | 0.251 | 0.641 | 0.605 | 0.745 | 0.667 | 0.271 | 0.102 | 0.723 | 0.839 | 0.607 |
| Left | Pericalcarine | 0.900 | 0.628 | 0.699 | 0.599 | 0.868 | 0.786 | 0.604 | 0.170 | 0.843 | 0.532 |
| Left | Postcentral | 0.478 | 0.022 | 0.039 | 0.032 | 0.730 | 0.529 | 0.545 | 0.235 | 0.585 | 0.219 |
| Left | Posterior Cingulate | 0.347 | 0.830 | 0.856 | 0.750 | 0.834 | 0.323 | 0.779 | 0.432 | 0.846 | 0.112 |
| Left | Precentral | 0.422 | 0.553 | 0.512 | 0.690 | 0.122 | 0.351 | 0.617 | 0.353 | 0.784 | 0.381 |
| Left | Precuneus | 0.003 | 0.003 | 0.004 | 0.015 | 0.778 | 0.258 | 0.343 | 0.929 | 0.943 | 0.309 |
| Left | Rostral Anterior Cingulate | 0.349 | 0.231 | 0.108 | 0.582 | 0.019 | 0.867 | 0.999 | 0.198 | 0.008 | 0.677 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Left | Rostral Middle Frontal | 0.244 | 0.197 | 0.185 | 0.336 | 0.051 | 0.674 | 0.044 | 0.890 | 0.898 | 0.476 |
| Left | Superior Frontal | 0.468 | 0.831 | 0.774 | 0.919 | 0.526 | 0.670 | 0.483 | 0.598 | 0.872 | 0.932 |
| Left | Superior Parietal | 0.011 | 0.042 | 0.113 | 0.029 | 0.544 | 0.359 | 0.162 | 0.341 | 0.726 | 0.130 |
| Left | Superior Temporal | 0.425 | 0.289 | 0.461 | 0.208 | 0.374 | 0.939 | 0.477 | 0.901 | 0.733 | 0.808 |
| Left | Supramarginal | 0.093 | 0.054 | 0.175 | 0.031 | 0.711 | 0.139 | 0.536 | 0.341 | 0.303 | 0.064 |
| Left | Temporal Pole | 0.869 | 0.175 | 0.084 | 0.567 | 0.569 | 0.574 | 0.635 | 0.947 | 0.615 | 0.793 |
| Left | Transverse Temporal | 0.443 | 0.443 | 0.703 | 0.270 | 0.227 | 0.908 | 0.374 | 0.610 | 0.914 | 0.068 |
| Right | Banks Superior Temporal Sulcus | 0.047 | 0.002 | 0.015 | 0.001 | 0.517 | 0.294 | 0.023 | 0.055 | 0.115 | 0.352 |
| Right | Caudal Anterior Cingulate | 0.006 | 0.193 | 0.547 | 0.058 | 0.479 | 0.089 | 0.077 | 0.027 | 0.898 | 0.455 |
| Right | Caudal Middle Frontal | 0.241 | 0.468 | 0.572 | 0.434 | 0.336 | 0.248 | 0.939 | 0.695 | 0.550 | 0.645 |
| Right | Cuneus | 0.008 | 0.002 | 0.004 | 0.005 | 0.962 | 0.008 | 0.078 | 0.090 | 0.575 | 0.037 |
| Right | Entorhinal | 0.729 | 0.385 | 0.594 | 0.289 | 0.947 | 0.309 | 0.342 | 0.218 | 0.276 | 0.354 |
| Right | Frontal Pole | 0.264 | 0.260 | 0.104 | 0.706 | 0.399 | 0.881 | 0.056 | 0.597 | 0.427 | 0.584 |
| Right | Fusiform | 0.181 | 0.638 | 0.800 | 0.554 | 0.664 | 0.965 | 0.172 | 0.570 | 0.926 | 0.682 |
| Right | Inferior Parietal | 0.172 | 0.007 | 0.049 | 0.002 | 0.505 | 0.126 | 0.798 | 0.576 | 0.322 | 0.313 |
| Right | Inferior Temporal | 0.556 | 0.696 | 0.863 | 0.604 | 0.983 | 0.251 | 0.292 | 0.830 | 0.943 | 0.291 |
| Right | Insula | 0.184 | 0.207 | 0.296 | 0.185 | 0.027 | 0.668 | 0.607 | 0.491 | 0.271 | 0.313 |
| Right | Isthmus Cingulate | 0.298 | 0.484 | 0.624 | 0.446 | 0.618 | 0.191 | 0.433 | 0.343 | 0.015 | 0.173 |
| Right | Lateral Occipital | 0.002 | 0.002 | 0.028 | 0.000 | 0.227 | 0.110 | 0.217 | 0.323 | 0.308 | 0.146 |
| Right | Lateral Orbitofrontal | 0.212 | 0.637 | 0.953 | 0.306 | 0.633 | 0.711 | 0.160 | 0.281 | 0.461 | 0.226 |
| Right | Lingual | 0.089 | 0.346 | 0.415 | 0.348 | 0.706 | 0.118 | 0.130 | 0.221 | 0.968 | 0.163 |
| Right | Medial Orbitofrontal | 0.082 | 0.626 | 0.493 | 0.847 | 0.931 | 0.559 | 0.868 | 0.954 | 0.164 | 0.755 |
| Right | Middle Temporal | 0.350 | 0.183 | 0.612 | 0.044 | 0.934 | 0.756 | 0.052 | 0.677 | 0.891 | 0.547 |
| Right | Paracentral | 0.021 | 0.227 | 0.663 | 0.059 | 0.199 | 0.290 | 0.013 | 0.123 | 0.607 | 0.502 |
| Right | Parahippocampal | 0.459 | 0.744 | 0.560 | 0.912 | 0.902 | 0.151 | 0.591 | 0.214 | 0.756 | 0.291 |
| Right | Pars Opercularis | 0.435 | 0.732 | 0.985 | 0.467 | 0.340 | 0.809 | 0.856 | 0.537 | 0.772 | 0.534 |
| Right | Pars Orbitalis | 0.836 | 0.189 | 0.491 | 0.072 | 0.342 | 0.835 | 0.867 | 0.609 | 0.700 | 0.792 |
| Right | Pars Triangularis | 0.614 | 0.813 | 0.975 | 0.631 | 0.316 | 0.923 | 0.536 | 0.625 | 0.396 | 0.685 |
| Right | Pericalcarine | 0.973 | 0.136 | 0.155 | 0.192 | 0.018 | 0.181 | 0.179 | 0.685 | 0.354 | 0.048 |
| Right | Postcentral | 0.129 | 0.105 | 0.238 | 0.066 | 0.290 | 0.754 | 0.293 | 0.785 | 0.392 | 0.287 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Right | Posterior Cingulate | 0.030 | 0.948 | 0.877 | 0.661 | 0.972 | 0.788 | 0.913 | 0.331 | 0.666 | 0.632 |
| Right | Precentral | 0.570 | 0.829 | 0.784 | 0.472 | 0.475 | 0.522 | 0.714 | 0.909 | 0.339 | 0.294 |
| Right | Precuneus | 0.002 | 0.032 | 0.040 | 0.076 | 0.727 | 0.203 | 0.341 | 0.453 | 0.891 | 0.295 |
| Right | Rostral Anterior Cingulate | 0.622 | 0.803 | 0.877 | 0.477 | 0.399 | 0.631 | 0.551 | 0.721 | 0.966 | 0.776 |
| Right | Rostral Middle Frontal | 0.150 | 0.255 | 0.273 | 0.344 | 0.192 | 0.377 | 0.060 | 0.785 | 0.867 | 0.524 |
| Right | Superior Frontal | 0.742 | 0.603 | 0.443 | 0.919 | 0.669 | 0.444 | 0.546 | 0.564 | 0.972 | 0.515 |
| Right | Superior Parietal | 0.061 | 0.050 | 0.131 | 0.033 | 0.562 | 0.236 | 0.322 | 0.336 | 0.560 | 0.479 |
| Right | Superior Temporal | 0.979 | 0.834 | 0.553 | 0.250 | 0.788 | 0.427 | 0.482 | 0.395 | 0.160 | 0.108 |
| Right | Supramarginal | 0.493 | 0.279 | 0.342 | 0.320 | 0.993 | 0.648 | 0.404 | 0.386 | 0.340 | 0.872 |
| Right | Temporal Pole | 0.146 | 0.562 | 0.995 | 0.273 | 0.738 | 0.355 | 0.088 | 0.410 | 0.738 | 0.413 |
| Right | Transverse Temporal | 0.331 | 0.990 | 0.782 | 0.704 | 0.265 | 0.286 | 0.421 | 0.236 | 0.377 | 0.049 |
| *Subcortex* | | | | | | | | | | | |
| Left | Accumbens | 0.219 | 0.676 | 0.687 | 0.736 | 0.256 | 0.373 | 0.309 | 0.363 | 0.240 | 0.850 |
| Left | Amygdala | 0.441 | 0.197 | 0.459 | 0.082 | 0.953 | 0.210 | 0.292 | 0.905 | 0.773 | 0.135 |
| Left | Caudate | 0.163 | 0.498 | 0.369 | 0.744 | 0.961 | 0.627 | 0.660 | 0.864 | 0.109 | 0.960 |
| Left | Hippocampus | 0.923 | 0.873 | 0.800 | 0.959 | 0.912 | 0.819 | 0.395 | 0.664 | 0.539 | 0.758 |
| Left | Pallidum | 0.740 | 0.224 | 0.198 | 0.360 | 0.502 | 0.150 | 0.958 | 0.271 | 0.164 | 0.091 |
| Left | Putamen | 0.654 | 0.775 | 0.933 | 0.647 | 0.735 | 0.353 | 0.524 | 0.406 | 0.586 | 0.790 |
| Left | Thalamus | 0.582 | 0.306 | 0.433 | 0.267 | 0.841 | 0.903 | 0.348 | 0.364 | 0.474 | 0.349 |
| Right | Accumbens | 0.169 | 0.460 | 0.327 | 0.730 | 0.427 | 0.610 | 0.495 | 0.353 | 0.728 | 0.708 |
| Right | Amygdala | 0.347 | 0.692 | 0.905 | 0.356 | 0.713 | 0.375 | 0.402 | 0.502 | 0.644 | 0.421 |
| Right | Caudate | 0.569 | 0.841 | 0.730 | 0.969 | 0.724 | 0.863 | 0.796 | 0.738 | 0.069 | 0.807 |
| Right | Hippocampus | 0.732 | 0.289 | 0.454 | 0.200 | 0.681 | 0.796 | 0.394 | 0.340 | 0.799 | 0.801 |
| Right | Pallidum | 0.626 | 0.228 | 0.280 | 0.267 | 0.336 | 0.207 | 0.864 | 0.219 | 0.209 | 0.049 |
| Right | Putamen | 0.803 | 0.766 | 0.849 | 0.722 | 0.700 | 0.239 | 0.489 | 0.256 | 0.453 | 0.359 |
| Right | Thalamus | 0.298 | 0.493 | 0.534 | 0.515 | 0.927 | 0.603 | 0.533 | 0.385 | 0.944 | 0.208 |

TEST = testosterone, E2 = estradiol

Table S4. *Unstandardized effects of age and puberty*

| Hemi | Region | Age B | Age SE | Puberty B | Puberty SE | Adrenal B | Adrenal SE | Gonadal B | Gonadal SE |
|---|---|---|---|---|---|---|---|---|---|
| *Cortex* | | | | | | | | | |
| Left | Banks Superior Temporal Sulcus | -0.037 | 0.02 | -0.034 | 0.019 | -0.029 | 0.016 | -0.026 | 0.019 |
| Left | Caudal Anterior Cingulate | -0.027 | 0.023 | -0.039 | 0.021 | -0.033 | 0.018 | -0.032 | 0.021 |
| Left | Caudal Middle Frontal | 0.003 | 0.013 | -0.014 | 0.012 | -0.015 | 0.01 | -0.006 | 0.012 |
| Left | Cuneus | -0.03 | 0.015 | -0.041 | 0.014 | -0.025 | 0.012 | -0.048 | 0.013 |
| Left | Entorhinal | -0.021 | 0.027 | -0.007 | 0.025 | -0.001 | 0.021 | -0.01 | 0.025 |
| Left | Frontal Pole | 0.029 | 0.027 | -0.003 | 0.025 | -0.006 | 0.021 | 0.002 | 0.025 |
| Left | Fusiform | -0.002 | 0.013 | 0.001 | 0.012 | 0.004 | 0.01 | -0.003 | 0.012 |
| Left | Inferior Parietal | -0.036 | 0.013 | -0.033 | 0.012 | -0.023 | 0.01 | -0.033 | 0.012 |
| Left | Inferior Temporal | -0.031 | 0.015 | -0.005 | 0.014 | -0.003 | 0.012 | -0.006 | 0.014 |
| Left | Insula | -0.015 | 0.019 | -0.013 | 0.017 | -0.006 | 0.015 | -0.017 | 0.017 |
| Left | Isthmus Cingulate | -0.035 | 0.02 | 0.002 | 0.019 | 0.003 | 0.016 | -0.001 | 0.019 |
| Left | Lateral Occipital | -0.027 | 0.014 | -0.029 | 0.013 | -0.019 | 0.011 | -0.032 | 0.012 |
| Left | Lateral Orbitofrontal | -0.02 | 0.013 | -0.007 | 0.012 | -0.013 | 0.01 | 0.004 | 0.012 |
| Left | Lingual | -0.01 | 0.015 | -0.016 | 0.013 | -0.008 | 0.011 | -0.022 | 0.013 |
| Left | Medial Orbitofrontal | -0.011 | 0.013 | -0.002 | 0.012 | -0.006 | 0.011 | 0.003 | 0.012 |
| Left | Middle Temporal | -0.023 | 0.016 | -0.013 | 0.015 | -0.005 | 0.013 | -0.017 | 0.015 |
| Left | Paracentral | -0.014 | 0.017 | -0.008 | 0.016 | -0.005 | 0.014 | -0.008 | 0.016 |
| Left | Parahippocampal | -0.038 | 0.031 | 0.031 | 0.029 | 0.032 | 0.024 | 0.016 | 0.029 |
| Left | Pars Opercularis | 0.002 | 0.014 | 0.01 | 0.013 | 0.007 | 0.011 | 0.011 | 0.013 |
| Left | Pars Orbitalis | -0.004 | 0.02 | -0.014 | 0.018 | -0.009 | 0.015 | -0.015 | 0.018 |
| Left | Pars Triangularis | -0.018 | 0.015 | -0.007 | 0.014 | -0.006 | 0.012 | -0.005 | 0.014 |
| Left | Pericalcarine | 0.002 | 0.015 | -0.007 | 0.014 | -0.005 | 0.012 | -0.007 | 0.014 |
| Left | Postcentral | -0.011 | 0.015 | -0.032 | 0.014 | -0.025 | 0.012 | -0.03 | 0.014 |
| Left | Posterior Cingulate | -0.016 | 0.017 | -0.003 | 0.016 | -0.002 | 0.013 | -0.005 | 0.016 |
| Left | Precentral | 0.011 | 0.014 | -0.008 | 0.013 | -0.007 | 0.011 | -0.005 | 0.013 |
| Left | Precuneus | -0.034 | 0.011 | -0.031 | 0.01 | -0.026 | 0.009 | -0.026 | 0.01 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Left | Rostral Anterior Cingulate | -0.02 | 0.022 | -0.024 | 0.02 | -0.027 | 0.017 | -0.011 | 0.02 |
| Left | Rostral Middle Frontal | -0.014 | 0.012 | -0.014 | 0.011 | -0.012 | 0.009 | -0.011 | 0.011 |
| Left | Superior Frontal | -0.01 | 0.014 | 0.003 | 0.013 | 0.003 | 0.011 | 0.001 | 0.013 |
| Left | Superior Parietal | -0.035 | 0.014 | -0.026 | 0.013 | -0.017 | 0.011 | -0.028 | 0.013 |
| Left | Superior Temporal | -0.013 | 0.016 | -0.016 | 0.015 | -0.01 | 0.013 | -0.019 | 0.015 |
| Left | Supramarginal | -0.024 | 0.014 | -0.025 | 0.013 | -0.015 | 0.011 | -0.028 | 0.013 |
| Left | Temporal Pole | 0.006 | 0.033 | -0.042 | 0.031 | -0.045 | 0.026 | -0.017 | 0.03 |
| Left | Transverse Temporal | -0.02 | 0.025 | -0.018 | 0.023 | -0.008 | 0.02 | -0.026 | 0.023 |
| Right | Banks Superior Temporal Sulcus | -0.036 | 0.018 | -0.052 | 0.016 | -0.035 | 0.014 | -0.055 | 0.016 |
| Right | Caudal Anterior Cingulate | -0.057 | 0.02 | -0.025 | 0.019 | -0.01 | 0.016 | -0.036 | 0.019 |
| Right | Caudal Middle Frontal | 0.017 | 0.014 | 0.009 | 0.013 | 0.006 | 0.011 | 0.01 | 0.013 |
| Right | Cuneus | -0.043 | 0.016 | -0.046 | 0.015 | -0.037 | 0.012 | -0.041 | 0.015 |
| Right | Entorhinal | 0.012 | 0.036 | 0.029 | 0.033 | 0.015 | 0.028 | 0.035 | 0.033 |
| Right | Frontal Pole | -0.031 | 0.028 | -0.029 | 0.026 | -0.036 | 0.022 | -0.01 | 0.026 |
| Right | Fusiform | -0.02 | 0.015 | -0.006 | 0.014 | -0.003 | 0.012 | -0.008 | 0.013 |
| Right | Inferior Parietal | -0.015 | 0.011 | -0.028 | 0.01 | -0.017 | 0.009 | -0.031 | 0.01 |
| Right | Inferior Temporal | -0.01 | 0.017 | -0.006 | 0.015 | -0.002 | 0.013 | -0.008 | 0.015 |
| Right | Insula | -0.025 | 0.019 | -0.022 | 0.017 | -0.015 | 0.015 | -0.023 | 0.017 |
| Right | Isthmus Cingulate | -0.022 | 0.021 | 0.013 | 0.019 | 0.008 | 0.016 | 0.014 | 0.019 |
| Right | Lateral Occipital | -0.043 | 0.014 | -0.04 | 0.013 | -0.025 | 0.011 | -0.045 | 0.013 |
| Right | Lateral Orbitofrontal | -0.019 | 0.015 | 0.007 | 0.014 | -0.001 | 0.012 | 0.014 | 0.014 |
| Right | Lingual | -0.024 | 0.014 | -0.012 | 0.013 | -0.009 | 0.011 | -0.012 | 0.013 |
| Right | Medial Orbitofrontal | -0.027 | 0.016 | -0.007 | 0.015 | -0.009 | 0.012 | -0.003 | 0.014 |
| Right | Middle Temporal | -0.014 | 0.015 | -0.018 | 0.014 | -0.006 | 0.012 | -0.028 | 0.014 |
| Right | Paracentral | -0.038 | 0.016 | -0.019 | 0.015 | -0.006 | 0.013 | -0.029 | 0.015 |
| Right | Parahippocampal | -0.021 | 0.029 | 0.009 | 0.027 | 0.013 | 0.023 | -0.003 | 0.026 |
| Right | Pars Opercularis | 0.012 | 0.015 | -0.005 | 0.014 | 0 | 0.012 | -0.01 | 0.014 |
| Right | Pars Orbitalis | 0.004 | 0.02 | 0.024 | 0.018 | 0.011 | 0.016 | 0.033 | 0.018 |
| Right | Pars Triangularis | 0.007 | 0.014 | -0.003 | 0.013 | 0 | 0.011 | -0.006 | 0.012 |
| Right | Pericalcarine | -0.001 | 0.017 | -0.023 | 0.016 | -0.019 | 0.013 | -0.02 | 0.015 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Right | Postcentral | -0.019 | 0.012 | -0.018 | 0.011 | -0.011 | 0.01 | -0.021 | 0.011 |
| Right | Posterior Cingulate | -0.035 | 0.016 | -0.001 | 0.015 | 0.002 | 0.013 | -0.006 | 0.015 |
| Right | Precentral | 0.007 | 0.013 | -0.003 | 0.012 | 0.003 | 0.01 | -0.008 | 0.012 |
| Right | Precuneus | -0.037 | 0.012 | -0.024 | 0.011 | -0.02 | 0.009 | -0.02 | 0.011 |
| Right | Rostral Anterior Cingulate | -0.011 | 0.022 | -0.005 | 0.021 | 0.003 | 0.018 | -0.015 | 0.02 |
| Right | Rostral Middle Frontal | -0.016 | 0.011 | -0.011 | 0.01 | -0.009 | 0.008 | -0.009 | 0.01 |
| Right | Superior Frontal | -0.004 | 0.013 | 0.006 | 0.012 | 0.008 | 0.01 | 0.001 | 0.012 |
| Right | Superior Parietal | -0.024 | 0.013 | -0.023 | 0.012 | -0.015 | 0.01 | -0.025 | 0.012 |
| Right | Superior Temporal | 0 | 0.016 | -0.003 | 0.015 | 0.008 | 0.013 | -0.017 | 0.015 |
| Right | Supramarginal | -0.009 | 0.012 | -0.012 | 0.011 | -0.009 | 0.01 | -0.011 | 0.011 |
| Right | Temporal Pole | -0.044 | 0.03 | -0.016 | 0.028 | 0 | 0.024 | -0.03 | 0.027 |
| Right | Transverse Temporal | 0.024 | 0.025 | 0 | 0.023 | 0.005 | 0.019 | -0.009 | 0.023 |
| *Subcortex* | | | | | | | | | |
| Left | Accumbens | -11.828 | 9.56 | -3.715 | 8.856 | -3.044 | 7.521 | -2.97 | 8.781 |
| Left | Amygdala | 12.59 | 16.291 | 19.374 | 14.926 | 9.463 | 12.741 | 25.835 | 14.702 |
| Left | Caudate | -74.479 | 53.084 | -33.479 | 49.206 | -37.639 | 41.721 | -16.003 | 48.85 |
| Left | Hippocampus | -3.815 | 39.161 | -5.776 | 36.052 | -7.777 | 30.611 | -1.85 | 35.738 |
| Left | Pallidum | -7.781 | 23.433 | 26.194 | 21.441 | 23.572 | 18.193 | 19.606 | 21.313 |
| Left | Putamen | -29.72 | 66.088 | 17.439 | 60.88 | 4.369 | 51.719 | 27.703 | 60.308 |
| Left | Thalamus | 41.044 | 74.367 | -70.188 | 68.237 | -45.654 | 58.064 | -75.372 | 67.578 |
| Right | Accumbens | -12.907 | 9.32 | -6.408 | 8.634 | -7.207 | 7.318 | -2.97 | 8.574 |
| Right | Amygdala | 18.744 | 19.853 | 7.278 | 18.34 | -1.86 | 15.585 | 16.785 | 18.12 |
| Right | Caudate | -29.354 | 51.396 | -9.516 | 47.382 | -13.905 | 40.223 | -1.806 | 46.972 |
| Right | Hippocampus | -13.621 | 39.695 | -38.789 | 36.379 | -23.253 | 30.974 | -46.387 | 35.973 |
| Right | Pallidum | 10.682 | 21.878 | 24.258 | 20.032 | 18.494 | 17.034 | 22.189 | 19.875 |
| Right | Putamen | -15.95 | 63.865 | 17.558 | 58.793 | 9.559 | 49.941 | 20.791 | 58.264 |
| Right | Thalamus | 75.704 | 72.428 | -46.04 | 66.87 | -35.407 | 56.81 | -43.268 | 66.295 |

B = unstandardized coefficients, SE = standard errors

Table S5. *Model fit comparisons examining unique variance explained by saliva over hair*

| Hemi | Region | ***DHEA*** | | | | ***TEST*** | | | | ***E2*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Chisq* | *Df* | *P val* | *AIC* | *Chisq* | *Df* | *P val* | *AIC* | *Chisq* | *Df* | *P val* | *AIC* |
| *Cortex* | | | | | | | | | | | | | |
| Left | Banks Superior Temporal Sulcus | 0.954 | 1 | 0.329 | 1.050 | 0.189 | 1 | 0.664 | 1.810 | 0.007 | 1 | 0.935 | 1.990 |
| Left | Caudal Anterior Cingulate | 0.006 | 1 | 0.939 | 1.990 | 0.352 | 1 | 0.553 | 1.650 | 0.278 | 1 | 0.598 | 1.720 |
| Left | Caudal Middle Frontal | 0.006 | 1 | 0.940 | 1.990 | 0.315 | 1 | 0.574 | 1.680 | 1.232 | 1 | 0.267 | 0.770 |
| Left | Cuneus | 0.015 | 1 | 0.903 | 1.980 | 0.243 | 1 | 0.622 | 1.760 | 0.048 | 1 | 0.827 | 1.950 |
| Left | Entorhinal | 0.092 | 1 | 0.762 | 1.910 | 0.043 | 1 | 0.836 | 1.960 | 0.008 | 1 | 0.927 | 1.990 |
| Left | Frontal Pole | 0.013 | 1 | 0.911 | 1.990 | 0.000 | 1 | 0.993 | 2.000 | 0.462 | 1 | 0.497 | 1.540 |
| Left | Fusiform | 1.923 | 1 | 0.166 | 0.080 | 3.208 | 1 | 0.073 | -1.210 | 2.191 | 1 | 0.139 | -0.190 |
| Left | Inferior Parietal | 3.620 | 1 | 0.057 | -1.620 | 5.536 | 1 | 0.019 | -3.540 | 1.357 | 1 | 0.244 | 0.640 |
| Left | Inferior Temporal | 0.260 | 1 | 0.610 | 1.740 | 0.020 | 1 | 0.887 | 1.980 | 0.230 | 1 | 0.632 | 1.770 |
| Left | Insula | 0.021 | 1 | 0.884 | 1.980 | 0.326 | 1 | 0.568 | 1.670 | 0.025 | 1 | 0.874 | 1.970 |
| Left | Isthmus Cingulate | 0.349 | 1 | 0.555 | 1.650 | 0.835 | 1 | 0.361 | 1.160 | 0.858 | 1 | 0.354 | 1.140 |
| Left | Lateral Occipital | 0.441 | 1 | 0.507 | 1.560 | 0.292 | 1 | 0.589 | 1.710 | 0.027 | 1 | 0.869 | 1.970 |
| Left | Lateral Orbitofrontal | 0.050 | 1 | 0.823 | 1.950 | 0.182 | 1 | 0.669 | 1.820 | 0.000 | 1 | 0.984 | 2.000 |
| Left | Lingual | 0.180 | 1 | 0.672 | 1.820 | 0.028 | 1 | 0.867 | 1.970 | 0.123 | 1 | 0.726 | 1.880 |
| Left | Medial Orbitofrontal | 0.961 | 1 | 0.327 | 1.040 | 0.714 | 1 | 0.398 | 1.290 | 4.681 | 1 | 0.030 | -2.680 |
| Left | Middle Temporal | 0.002 | 1 | 0.965 | 2.000 | 0.198 | 1 | 0.656 | 1.800 | 0.363 | 1 | 0.547 | 1.640 |
| Left | Paracentral | 1.158 | 1 | 0.282 | 0.840 | 0.903 | 1 | 0.342 | 1.100 | 0.015 | 1 | 0.903 | 1.990 |
| Left | Parahippocampal | 2.348 | 1 | 0.125 | -0.350 | 0.790 | 1 | 0.374 | 1.210 | 1.016 | 1 | 0.313 | 0.980 |
| Left | Pars Opercularis | 0.001 | 1 | 0.975 | 2.000 | 1.135 | 1 | 0.287 | 0.870 | 0.725 | 1 | 0.395 | 1.280 |
| Left | Pars Orbitalis | 0.126 | 1 | 0.723 | 1.870 | 0.257 | 1 | 0.612 | 1.740 | 0.086 | 1 | 0.769 | 1.910 |
| Left | Pars Triangularis | 1.231 | 1 | 0.267 | 0.770 | 0.248 | 1 | 0.618 | 1.750 | 1.015 | 1 | 0.314 | 0.980 |
| Left | Pericalcarine | 0.074 | 1 | 0.785 | 1.930 | 0.375 | 1 | 0.541 | 1.630 | 1.653 | 1 | 0.199 | 0.350 |
| Left | Postcentral | 0.401 | 1 | 0.527 | 1.600 | 1.401 | 1 | 0.237 | 0.600 | 1.114 | 1 | 0.291 | 0.890 |
| Left | Posterior Cingulate | 0.996 | 1 | 0.318 | 1.000 | 2.538 | 1 | 0.111 | -0.540 | 0.551 | 1 | 0.458 | 1.450 |
| Left | Precentral | 0.950 | 1 | 0.330 | 1.050 | 0.862 | 1 | 0.353 | 1.140 | 0.663 | 1 | 0.415 | 1.340 |
| Left | Precuneus | 1.318 | 1 | 0.251 | 0.680 | 1.056 | 1 | 0.304 | 0.940 | 0.068 | 1 | 0.795 | 1.930 |
| Left | Rostral Anterior Cingulate | 0.042 | 1 | 0.838 | 1.960 | 0.619 | 1 | 0.432 | 1.380 | 1.928 | 1 | 0.165 | 0.070 |
| Left | Rostral Middle Frontal | 0.165 | 1 | 0.684 | 1.830 | 0.553 | 1 | 0.457 | 1.450 | 0.374 | 1 | 0.541 | 1.630 |
| Left | Superior Frontal | 0.193 | 1 | 0.660 | 1.810 | 0.004 | 1 | 0.947 | 2.000 | 0.697 | 1 | 0.404 | 1.300 |
| Left | Superior Parietal | 0.878 | 1 | 0.349 | 1.120 | 2.249 | 1 | 0.134 | -0.250 | 0.256 | 1 | 0.613 | 1.740 |
| Left | Superior Temporal | 0.008 | 1 | 0.929 | 1.990 | 0.042 | 1 | 0.838 | 1.960 | 0.162 | 1 | 0.687 | 1.840 |
| Left | Supramarginal | 2.260 | 1 | 0.133 | -0.260 | 3.100 | 1 | 0.078 | -1.100 | 0.633 | 1 | 0.426 | 1.370 |
| Left | Temporal Pole | 0.316 | 1 | 0.574 | 1.680 | 0.110 | 1 | 0.740 | 1.890 | 0.011 | 1 | 0.915 | 1.990 |
| Left | Transverse Temporal | 0.018 | 1 | 0.894 | 1.980 | 3.508 | 1 | 0.061 | -1.510 | 0.788 | 1 | 0.375 | 1.210 |
| Right | Banks Superior Temporal Sulcus | 1.151 | 1 | 0.283 | 0.850 | 0.571 | 1 | 0.450 | 1.430 | 1.543 | 1 | 0.214 | 0.460 |
| Right | Caudal Anterior Cingulate | 2.941 | 1 | 0.086 | -0.940 | 0.554 | 1 | 0.457 | 1.450 | 3.001 | 1 | 0.083 | -1.000 |
| Right | Caudal Middle Frontal | 1.408 | 1 | 0.235 | 0.590 | 0.155 | 1 | 0.694 | 1.850 | 0.205 | 1 | 0.651 | 1.800 |
| Right | Cuneus | 7.215 | 1 | 0.007 | -5.210 | 4.241 | 1 | 0.039 | -2.240 | 1.395 | 1 | 0.238 | 0.600 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Right | Entorhinal | 1.058 | 1 | 0.304 | 0.940 | 0.657 | 1 | 0.418 | 1.340 | 0.950 | 1 | 0.330 | 1.050 |
| Right | Frontal Pole | 0.020 | 1 | 0.888 | 1.980 | 0.437 | 1 | 0.509 | 1.560 | 0.021 | 1 | 0.884 | 1.980 |
| Right | Fusiform | 0.001 | 1 | 0.970 | 2.000 | 0.165 | 1 | 0.684 | 1.830 | 1.291 | 1 | 0.256 | 0.710 |
| Right | Inferior Parietal | 2.373 | 1 | 0.123 | -0.370 | 0.820 | 1 | 0.365 | 1.180 | 0.258 | 1 | 0.612 | 1.740 |
| Right | Inferior Temporal | 1.346 | 1 | 0.246 | 0.650 | 1.139 | 1 | 0.286 | 0.860 | 0.397 | 1 | 0.529 | 1.600 |
| Right | Insula | 0.171 | 1 | 0.679 | 1.830 | 1.383 | 1 | 0.240 | 0.620 | 0.303 | 1 | 0.582 | 1.700 |
| Right | Isthmus Cingulate | 1.774 | 1 | 0.183 | 0.230 | 3.051 | 1 | 0.081 | -1.050 | 0.533 | 1 | 0.465 | 1.470 |
| Right | Lateral Occipital | 2.703 | 1 | 0.100 | -0.700 | 2.627 | 1 | 0.105 | -0.630 | 0.369 | 1 | 0.544 | 1.630 |
| Right | Lateral Orbitofrontal | 0.146 | 1 | 0.702 | 1.850 | 1.777 | 1 | 0.183 | 0.220 | 2.933 | 1 | 0.087 | -0.930 |
| Right | Lingual | 2.481 | 1 | 0.115 | -0.480 | 2.037 | 1 | 0.153 | -0.040 | 0.577 | 1 | 0.447 | 1.420 |
| Right | Medial Orbitofrontal | 0.351 | 1 | 0.554 | 1.650 | 0.020 | 1 | 0.887 | 1.980 | 0.000 | 1 | 0.999 | 2.000 |
| Right | Middle Temporal | 0.099 | 1 | 0.752 | 1.900 | 0.356 | 1 | 0.551 | 1.640 | 0.078 | 1 | 0.781 | 1.920 |
| Right | Paracentral | 1.126 | 1 | 0.289 | 0.870 | 0.384 | 1 | 0.536 | 1.620 | 0.577 | 1 | 0.447 | 1.420 |
| Right | Parahippocampal | 2.107 | 1 | 0.147 | -0.110 | 1.250 | 1 | 0.264 | 0.750 | 2.392 | 1 | 0.122 | -0.390 |
| Right | Pars Opercularis | 0.067 | 1 | 0.796 | 1.930 | 0.452 | 1 | 0.502 | 1.550 | 0.357 | 1 | 0.550 | 1.640 |
| Right | Pars Orbitalis | 0.039 | 1 | 0.843 | 1.960 | 0.102 | 1 | 0.750 | 1.900 | 0.240 | 1 | 0.624 | 1.760 |
| Right | Pars Triangularis | 0.013 | 1 | 0.911 | 1.990 | 0.093 | 1 | 0.760 | 1.910 | 0.581 | 1 | 0.446 | 1.420 |
| Right | Pericalcarine | 1.837 | 1 | 0.175 | 0.160 | 3.612 | 1 | 0.057 | -1.610 | 0.004 | 1 | 0.948 | 2.000 |
| Right | Postcentral | 0.093 | 1 | 0.761 | 1.910 | 0.958 | 1 | 0.328 | 1.040 | 0.010 | 1 | 0.920 | 1.990 |
| Right | Posterior Cingulate | 0.074 | 1 | 0.786 | 1.930 | 0.295 | 1 | 0.587 | 1.700 | 1.017 | 1 | 0.313 | 0.980 |
| Right | Precentral | 0.434 | 1 | 0.510 | 1.570 | 0.905 | 1 | 0.341 | 1.100 | 0.068 | 1 | 0.794 | 1.930 |
| Right | Precuneus | 1.647 | 1 | 0.199 | 0.350 | 1.102 | 1 | 0.294 | 0.900 | 0.205 | 1 | 0.650 | 1.790 |
| Right | Rostral Anterior Cingulate | 0.227 | 1 | 0.633 | 1.770 | 0.087 | 1 | 0.768 | 1.910 | 0.026 | 1 | 0.871 | 1.970 |
| Right | Rostral Middle Frontal | 0.781 | 1 | 0.377 | 1.220 | 0.395 | 1 | 0.530 | 1.610 | 1.027 | 1 | 0.311 | 0.970 |
| Right | Superior Frontal | 0.608 | 1 | 0.435 | 1.390 | 0.435 | 1 | 0.510 | 1.570 | 0.157 | 1 | 0.692 | 1.840 |
| Right | Superior Parietal | 1.423 | 1 | 0.233 | 0.580 | 0.419 | 1 | 0.517 | 1.580 | 0.448 | 1 | 0.503 | 1.550 |
| Right | Superior Temporal | 0.652 | 1 | 0.419 | 1.350 | 2.155 | 1 | 0.142 | -0.160 | 1.406 | 1 | 0.236 | 0.590 |
| Right | Supramarginal | 0.213 | 1 | 0.644 | 1.790 | 0.002 | 1 | 0.967 | 2.000 | 1.577 | 1 | 0.209 | 0.420 |
| Right | Temporal Pole | 0.866 | 1 | 0.352 | 1.130 | 0.769 | 1 | 0.380 | 1.230 | 0.065 | 1 | 0.799 | 1.940 |
| Right | Transverse Temporal | 1.146 | 1 | 0.284 | 0.850 | 3.613 | 1 | 0.057 | -1.610 | 0.955 | 1 | 0.328 | 1.040 |
| *Subcortex* | | | | | | | | | | | | | |
| Left | Accumbens | 0.793 | 1 | 0.373 | 1.210 | 0.002 | 1 | 0.965 | 2.000 | 0.363 | 1 | 0.547 | 1.640 |
| Left | Amygdala | 1.610 | 1 | 0.205 | 0.390 | 2.212 | 1 | 0.137 | -0.210 | 0.278 | 1 | 0.598 | 1.720 |
| Left | Caudate | 0.242 | 1 | 0.623 | 1.760 | 0.024 | 1 | 0.877 | 1.980 | 0.000 | 1 | 0.985 | 2.000 |
| Left | Hippocampus | 0.055 | 1 | 0.815 | 1.950 | 0.055 | 1 | 0.814 | 1.940 | 0.626 | 1 | 0.429 | 1.370 |
| Left | Pallidum | 2.151 | 1 | 0.143 | -0.150 | 3.699 | 1 | 0.054 | -1.700 | 1.460 | 1 | 0.227 | 0.540 |
| Left | Putamen | 0.891 | 1 | 0.345 | 1.110 | 0.116 | 1 | 0.733 | 1.880 | 0.434 | 1 | 0.510 | 1.570 |
| Left | Thalamus | 0.016 | 1 | 0.900 | 1.980 | 1.103 | 1 | 0.294 | 0.900 | 0.396 | 1 | 0.529 | 1.600 |
| Right | Accumbens | 0.256 | 1 | 0.613 | 1.740 | 0.114 | 1 | 0.736 | 1.890 | 0.558 | 1 | 0.455 | 1.440 |
| Right | Amygdala | 0.798 | 1 | 0.372 | 1.200 | 0.580 | 1 | 0.446 | 1.420 | 1.089 | 1 | 0.297 | 0.910 |
| Right | Caudate | 0.032 | 1 | 0.858 | 1.970 | 0.238 | 1 | 0.626 | 1.760 | 0.070 | 1 | 0.791 | 1.930 |
| Right | Hippocampus | 0.071 | 1 | 0.790 | 1.930 | 0.084 | 1 | 0.772 | 1.920 | 1.842 | 1 | 0.175 | 0.160 |
| Right | Pallidum | 1.678 | 1 | 0.195 | 0.320 | 4.795 | 1 | 0.029 | -2.790 | 1.595 | 1 | 0.207 | 0.400 |
| Right | Putamen | 1.433 | 1 | 0.231 | 0.570 | 1.069 | 1 | 0.301 | 0.930 | 0.933 | 1 | 0.334 | 1.070 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Right | Thalamus | 0.275 | 1 | 0.600 | 1.720 | 1.627 | 1 | 0.202 | 0.370 | 0.494 | 1 | 0.482 | 1.510 |

Models examine unique variance explained by saliva after accounting for hair. TEST = testosterone, E2 = estradiol

Table S6. *Model fit comparisons examining unique variance explained by hair over saliva*

| **Hemi** | **Region** | ***DHEA*** | | | | ***TEST*** | | | | ***E2*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Chisq* | *Df* | *P val* | *AIC* | *Chisq* | *Df* | *P val* | *AIC* | *Chisq* | *Df* | *P val* | *AIC* |
| *Cortex* | | | | | | | | | | | | | |
| Left | Banks Superior Temporal Sulcus | 0.498 | 1 | 0.480 | 1.500 | 0.022 | 1 | 0.883 | 1.980 | 3.817 | 1 | 0.051 | -1.820 |
| Left | Caudal Anterior Cingulate | 1.262 | 1 | 0.261 | 0.740 | 1.630 | 1 | 0.202 | 0.370 | 0.093 | 1 | 0.760 | 1.910 |
| Left | Caudal Middle Frontal | 0.678 | 1 | 0.410 | 1.320 | 1.210 | 1 | 0.271 | 0.790 | 1.235 | 1 | 0.266 | 0.770 |
| Left | Cuneus | 0.363 | 1 | 0.547 | 1.640 | 0.225 | 1 | 0.635 | 1.780 | 1.643 | 1 | 0.200 | 0.360 |
| Left | Entorhinal | 0.706 | 1 | 0.401 | 1.290 | 0.003 | 1 | 0.957 | 2.000 | 1.079 | 1 | 0.299 | 0.920 |
| Left | Frontal Pole | 0.408 | 1 | 0.523 | 1.590 | 0.813 | 1 | 0.367 | 1.190 | 0.024 | 1 | 0.877 | 1.980 |
| Left | Fusiform | 0.111 | 1 | 0.739 | 1.890 | 0.242 | 1 | 0.623 | 1.760 | 1.363 | 1 | 0.243 | 0.640 |
| Left | Inferior Parietal | 0.232 | 1 | 0.630 | 1.770 | 0.018 | 1 | 0.893 | 1.980 | 2.020 | 1 | 0.155 | -0.020 |
| Left | Inferior Temporal | 0.846 | 1 | 0.358 | 1.150 | 0.226 | 1 | 0.635 | 1.770 | 1.488 | 1 | 0.222 | 0.510 |
| Left | Insula | 3.769 | 1 | 0.052 | -1.770 | 0.211 | 1 | 0.646 | 1.790 | 0.249 | 1 | 0.618 | 1.750 |
| Left | Isthmus Cingulate | 0.633 | 1 | 0.426 | 1.370 | 3.677 | 1 | 0.055 | -1.680 | 1.461 | 1 | 0.227 | 0.540 |
| Left | Lateral Occipital | 0.656 | 1 | 0.418 | 1.340 | 0.004 | 1 | 0.950 | 2.000 | 0.390 | 1 | 0.532 | 1.610 |
| Left | Lateral Orbitofrontal | 0.157 | 1 | 0.692 | 1.840 | 0.311 | 1 | 0.577 | 1.690 | 0.604 | 1 | 0.437 | 1.400 |
| Left | Lingual | 0.000 | 1 | 0.992 | 2.000 | 0.001 | 1 | 0.971 | 2.000 | 2.424 | 1 | 0.119 | -0.420 |
| Left | Medial Orbitofrontal | 1.123 | 1 | 0.289 | 0.880 | 0.103 | 1 | 0.748 | 1.900 | 0.053 | 1 | 0.818 | 1.950 |
| Left | Middle Temporal | 2.283 | 1 | 0.131 | -0.280 | 0.564 | 1 | 0.453 | 1.440 | 4.304 | 1 | 0.038 | -2.300 |
| Left | Paracentral | 0.023 | 1 | 0.879 | 1.980 | 0.368 | 1 | 0.544 | 1.630 | 0.329 | 1 | 0.566 | 1.670 |
| Left | Parahippocampal | 0.262 | 1 | 0.609 | 1.740 | 0.025 | 1 | 0.875 | 1.980 | 1.906 | 1 | 0.167 | 0.090 |
| Left | Pars Opercularis | 5.374 | 1 | 0.020 | -3.370 | 0.019 | 1 | 0.891 | 1.980 | 0.021 | 1 | 0.885 | 1.980 |
| Left | Pars Orbitalis | 1.340 | 1 | 0.247 | 0.660 | 1.054 | 1 | 0.305 | 0.950 | 0.293 | 1 | 0.589 | 1.710 |
| Left | Pars Triangularis | 0.179 | 1 | 0.673 | 1.820 | 0.020 | 1 | 0.888 | 1.980 | 3.625 | 1 | 0.057 | -1.620 |
| Left | Pericalcarine | 0.027 | 1 | 0.869 | 1.970 | 0.015 | 1 | 0.902 | 1.980 | 0.002 | 1 | 0.962 | 2.000 |
| Left | Postcentral | 0.116 | 1 | 0.733 | 1.880 | 0.162 | 1 | 0.687 | 1.840 | 0.043 | 1 | 0.835 | 1.960 |
| Left | Posterior Cingulate | 0.040 | 1 | 0.842 | 1.960 | 0.000 | 1 | 0.997 | 2.000 | 0.000 | 1 | 0.993 | 2.000 |
| Left | Precentral | 2.503 | 1 | 0.114 | -0.500 | 0.155 | 1 | 0.694 | 1.850 | 0.037 | 1 | 0.847 | 1.960 |
| Left | Precuneus | 0.091 | 1 | 0.763 | 1.910 | 0.003 | 1 | 0.954 | 2.000 | 0.981 | 1 | 0.322 | 1.020 |
| Left | Rostral Anterior Cingulate | 5.670 | 1 | 0.017 | -3.670 | 7.728 | 1 | 0.005 | -5.730 | 0.237 | 1 | 0.626 | 1.760 |
| Left | Rostral Middle Frontal | 3.865 | 1 | 0.049 | -1.860 | 0.049 | 1 | 0.824 | 1.950 | 4.504 | 1 | 0.034 | -2.500 |
| Left | Superior Frontal | 0.420 | 1 | 0.517 | 1.580 | 0.024 | 1 | 0.878 | 1.980 | 0.917 | 1 | 0.338 | 1.080 |
| Left | Superior Parietal | 0.395 | 1 | 0.530 | 1.610 | 0.027 | 1 | 0.868 | 1.970 | 1.333 | 1 | 0.248 | 0.670 |
| Left | Superior Temporal | 0.812 | 1 | 0.368 | 1.190 | 0.100 | 1 | 0.752 | 1.900 | 0.663 | 1 | 0.416 | 1.340 |
| Left | Supramarginal | 0.159 | 1 | 0.690 | 1.840 | 0.682 | 1 | 0.409 | 1.320 | 0.097 | 1 | 0.755 | 1.900 |
| Left | Temporal Pole | 0.324 | 1 | 0.569 | 1.680 | 0.299 | 1 | 0.584 | 1.700 | 0.238 | 1 | 0.626 | 1.760 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Left | Transverse Temporal | 1.497 | 1 | 0.221 | 0.500 | 0.120 | 1 | 0.729 | 1.880 | 1.331 | 1 | 0.249 | 0.670 |
| Right | Banks Superior Temporal Sulcus | 0.452 | 1 | 0.502 | 1.550 | 2.221 | 1 | 0.136 | -0.220 | 3.045 | 1 | 0.081 | -1.050 |
| Right | Caudal Anterior Cingulate | 0.493 | 1 | 0.482 | 1.510 | 0.001 | 1 | 0.972 | 2.000 | 1.211 | 1 | 0.271 | 0.790 |
| Right | Caudal Middle Frontal | 0.988 | 1 | 0.320 | 1.010 | 0.303 | 1 | 0.582 | 1.700 | 0.053 | 1 | 0.818 | 1.950 |
| Right | Cuneus | 0.000 | 1 | 0.989 | 2.000 | 0.098 | 1 | 0.755 | 1.900 | 1.623 | 1 | 0.203 | 0.380 |
| Right | Entorhinal | 0.003 | 1 | 0.957 | 2.000 | 0.989 | 1 | 0.320 | 1.010 | 0.320 | 1 | 0.572 | 1.680 |
| Right | Frontal Pole | 0.726 | 1 | 0.394 | 1.270 | 0.777 | 1 | 0.378 | 1.220 | 3.478 | 1 | 0.062 | -1.480 |
| Right | Fusiform | 0.192 | 1 | 0.661 | 1.810 | 0.002 | 1 | 0.966 | 2.000 | 2.865 | 1 | 0.091 | -0.870 |
| Right | Inferior Parietal | 0.438 | 1 | 0.508 | 1.560 | 0.781 | 1 | 0.377 | 1.220 | 0.004 | 1 | 0.948 | 2.000 |
| Right | Inferior Temporal | 0.000 | 1 | 0.995 | 2.000 | 0.004 | 1 | 0.951 | 2.000 | 1.487 | 1 | 0.223 | 0.510 |
| Right | Insula | 4.962 | 1 | 0.026 | -2.960 | 1.580 | 1 | 0.209 | 0.420 | 0.088 | 1 | 0.767 | 1.910 |
| Right | Isthmus Cingulate | 0.276 | 1 | 0.599 | 1.720 | 7.178 | 1 | 0.007 | -5.180 | 0.241 | 1 | 0.624 | 1.760 |
| Right | Lateral Occipital | 1.580 | 1 | 0.209 | 0.420 | 1.531 | 1 | 0.216 | 0.470 | 0.926 | 1 | 0.336 | 1.070 |
| Right | Lateral Orbitofrontal | 0.239 | 1 | 0.625 | 1.760 | 0.833 | 1 | 0.362 | 1.170 | 3.766 | 1 | 0.052 | -1.770 |
| Right | Lingual | 0.133 | 1 | 0.715 | 1.870 | 0.049 | 1 | 0.825 | 1.950 | 1.388 | 1 | 0.239 | 0.610 |
| Right | Medial Orbitofrontal | 0.009 | 1 | 0.924 | 1.990 | 1.899 | 1 | 0.168 | 0.100 | 0.025 | 1 | 0.874 | 1.970 |
| Right | Middle Temporal | 0.008 | 1 | 0.930 | 1.990 | 0.004 | 1 | 0.950 | 2.000 | 3.753 | 1 | 0.053 | -1.750 |
| Right | Paracentral | 1.669 | 1 | 0.196 | 0.330 | 0.192 | 1 | 0.661 | 1.810 | 4.423 | 1 | 0.035 | -2.420 |
| Right | Parahippocampal | 0.011 | 1 | 0.915 | 1.990 | 0.206 | 1 | 0.650 | 1.790 | 1.110 | 1 | 0.292 | 0.890 |
| Right | Pars Opercularis | 0.936 | 1 | 0.333 | 1.060 | 0.141 | 1 | 0.708 | 1.860 | 0.001 | 1 | 0.971 | 2.000 |
| Right | Pars Orbitalis | 0.920 | 1 | 0.338 | 1.080 | 0.182 | 1 | 0.670 | 1.820 | 0.000 | 1 | 0.991 | 2.000 |
| Right | Pars Triangularis | 1.031 | 1 | 0.310 | 0.970 | 0.662 | 1 | 0.416 | 1.340 | 0.727 | 1 | 0.394 | 1.270 |
| Right | Pericalcarine | 5.738 | 1 | 0.017 | -3.740 | 0.498 | 1 | 0.480 | 1.500 | 1.680 | 1 | 0.195 | 0.320 |
| Right | Postcentral | 1.138 | 1 | 0.286 | 0.860 | 0.547 | 1 | 0.459 | 1.450 | 1.065 | 1 | 0.302 | 0.940 |
| Right | Posterior Cingulate | 0.001 | 1 | 0.975 | 2.000 | 0.251 | 1 | 0.616 | 1.750 | 0.061 | 1 | 0.804 | 1.940 |
| Right | Precentral | 0.538 | 1 | 0.463 | 1.460 | 0.713 | 1 | 0.398 | 1.290 | 0.193 | 1 | 0.661 | 1.810 |
| Right | Precuneus | 0.115 | 1 | 0.735 | 1.890 | 0.000 | 1 | 0.995 | 2.000 | 0.557 | 1 | 0.456 | 1.440 |
| Right | Rostral Anterior Cingulate | 0.717 | 1 | 0.397 | 1.280 | 0.006 | 1 | 0.937 | 1.990 | 0.259 | 1 | 0.611 | 1.740 |
| Right | Rostral Middle Frontal | 1.720 | 1 | 0.190 | 0.280 | 0.008 | 1 | 0.929 | 1.990 | 4.563 | 1 | 0.033 | -2.560 |
| Right | Superior Frontal | 0.197 | 1 | 0.657 | 1.800 | 0.002 | 1 | 0.962 | 2.000 | 0.189 | 1 | 0.664 | 1.810 |
| Right | Superior Parietal | 0.329 | 1 | 0.566 | 1.670 | 0.255 | 1 | 0.614 | 1.750 | 0.503 | 1 | 0.478 | 1.500 |
| Right | Superior Temporal | 0.080 | 1 | 0.777 | 1.920 | 1.541 | 1 | 0.215 | 0.460 | 1.172 | 1 | 0.279 | 0.830 |
| Right | Supramarginal | 0.000 | 1 | 0.988 | 2.000 | 0.908 | 1 | 0.341 | 1.090 | 1.521 | 1 | 0.217 | 0.480 |
| Right | Temporal Pole | 0.107 | 1 | 0.743 | 1.890 | 0.199 | 1 | 0.655 | 1.800 | 2.350 | 1 | 0.125 | -0.350 |
| Right | Transverse Temporal | 1.253 | 1 | 0.263 | 0.750 | 0.437 | 1 | 0.508 | 1.560 | 0.182 | 1 | 0.670 | 1.820 |
| *Subcortex* | | | | | | | | | | | | | |
| Left | Accumbens | 1.304 | 1 | 0.253 | 0.700 | 1.375 | 1 | 0.241 | 0.620 | 0.573 | 1 | 0.449 | 1.430 |
| Left | Amygdala | 0.006 | 1 | 0.939 | 1.990 | 0.011 | 1 | 0.917 | 1.990 | 1.399 | 1 | 0.237 | 0.600 |
| Left | Caudate | 0.003 | 1 | 0.956 | 2.000 | 2.654 | 1 | 0.103 | -0.650 | 0.168 | 1 | 0.682 | 1.830 |
| Left | Hippocampus | 0.013 | 1 | 0.908 | 1.990 | 0.345 | 1 | 0.557 | 1.660 | 1.173 | 1 | 0.279 | 0.830 |
| Left | Pallidum | 0.497 | 1 | 0.481 | 1.500 | 2.755 | 1 | 0.097 | -0.760 | 0.223 | 1 | 0.637 | 1.780 |
| Left | Putamen | 0.127 | 1 | 0.722 | 1.870 | 0.348 | 1 | 0.555 | 1.650 | 0.141 | 1 | 0.707 | 1.860 |
| Left | Thalamus | 0.042 | 1 | 0.838 | 1.960 | 0.727 | 1 | 0.394 | 1.270 | 0.453 | 1 | 0.501 | 1.550 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Right | Accumbens | 0.635 | 1 | 0.426 | 1.360 | 0.095 | 1 | 0.758 | 1.910 | 0.151 | 1 | 0.698 | 1.850 |
| Right | Amygdala | 0.130 | 1 | 0.718 | 1.870 | 0.137 | 1 | 0.712 | 1.860 | 1.345 | 1 | 0.246 | 0.650 |
| Right | Caudate | 0.129 | 1 | 0.719 | 1.870 | 3.552 | 1 | 0.059 | -1.550 | 0.024 | 1 | 0.878 | 1.980 |
| Right | Hippocampus | 0.175 | 1 | 0.675 | 1.820 | 0.085 | 1 | 0.770 | 1.910 | 1.655 | 1 | 0.198 | 0.340 |
| Right | Pallidum | 0.994 | 1 | 0.319 | 1.010 | 2.435 | 1 | 0.119 | -0.430 | 0.077 | 1 | 0.781 | 1.920 |
| Right | Putamen | 0.166 | 1 | 0.684 | 1.830 | 0.785 | 1 | 0.376 | 1.210 | 0.104 | 1 | 0.748 | 1.900 |
| Right | Thalamus | 0.007 | 1 | 0.931 | 1.990 | 0.008 | 1 | 0.929 | 1.990 | 0.121 | 1 | 0.728 | 1.880 |

Models examine unique variance explained by hair after accounting for saliva. TEST = testosterone, E2 = estradiol

Table S7. *Difference between partial correlations (controlling for pubertal stage) and Pearson's correlations*

| **Hemi** | **Region** | **DHEA_hair** | **DHEA_saliva** | **E2_hair** | **E2_saliva** | **TEST_hair** | **TEST_saliva** |
|---|---|---|---|---|---|---|---|
| Left | Accumbens | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 | 0.02 |
| Left | Amygdala | 0.02 | -0.04 | -0.03 | -0.06 | -0.02 | -0.04 |
| Left | Caudate | 0.00 | 0.03 | 0.01 | 0.03 | 0.00 | 0.02 |
| Left | Hippocampus | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| Left | Pallidum | 0.01 | -0.04 | -0.03 | -0.04 | -0.02 | -0.03 |
| Left | Putamen | 0.00 | -0.01 | 0.00 | -0.01 | -0.01 | -0.01 |
| Left | Thalamus | -0.01 | 0.05 | 0.03 | 0.05 | 0.01 | 0.05 |
| Right | Accumbens | 0.00 | 0.03 | 0.02 | 0.02 | 0.00 | 0.03 |
| Right | Amygdala | 0.00 | 0.00 | -0.01 | -0.03 | 0.00 | -0.01 |
| Right | Caudate | 0.00 | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 |
| Right | Hippocampus | -0.01 | 0.04 | 0.03 | 0.04 | 0.02 | 0.04 |
| Right | Pallidum | 0.01 | -0.04 | -0.03 | -0.04 | -0.01 | -0.04 |
| Right | Putamen | 0.01 | 0.00 | -0.01 | 0.00 | -0.01 | -0.01 |
| Right | Thalamus | -0.01 | 0.04 | 0.02 | 0.04 | 0.00 | 0.04 |

Values are calculated as Partial R - Pearson's R. Negative values indicate more negative or less positive correlations, and positive values indicate less negative or more positive correlations. TEST = testosterone, E2 = estradiol