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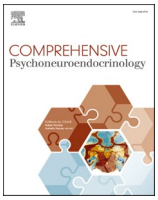

The effects of puberty and its hormones on subcortical brain development

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ABSTRACT

Puberty triggers a period of structural "re-organization" in the brain, when rising hormone levels act via receptors to influence morphology. However, our understanding of these neuroendocrine processes in humans remains poor. As such, the current longitudinal study characterized development of the human subcortex during puberty, including changes in relation to pubertal (Tanner) stage and hormone (testosterone, dehydroepiandrosterone [DHEA]) levels. Beyond normative group-level patterns of development, we also examined whether individual differences in the rate of pubertal maturation (i.e., "pubertal/hormonal tempo") were associated with variations in subcortical trajectories. Participants (N = 192; scans = 366) completed up to three waves of MRI assessments between 8.5 and 14.5 years of age. Parents completed questionnaire assessments of pubertal stage at each wave, and adolescents provided hormone samples on a subset of waves. Generalized additive mixture models were used to characterize trajectories of subcortical development. Results showed that development of most subcortical structures was related to pubertal stage, although findings were mostly non-significant when controlling for age. Testosterone and DHEA levels were related to development of the amygdala, hippocampus and pallidum in both sexes, and findings in the amygdala remained significant when controlling for age. Additionally, we found that variability in hormonal (specifically testosterone) tempo was related to right hippocampal development in males, with an accelerated pattern of hippocampal development in those with greater increases in testosterone levels. Overall, our findings suggest prominent hormonal influences on the amygdala and hippocampus, consistent with the prevalence of androgen and estrogen receptors in these regions. We speculate that these findings are most likely reflective of the important role of adrenarcheal processes on adolescent brain development.

1. Introduction

The transition from childhood to adolescence is characterized by significant brain maturation, particularly within regions that subserve developmentally salient cognitions and behaviors. This period begins with the onset of puberty, when the release of pubertal hormones triggers the process of sexual maturation. Animal research suggests that these hormones also influence brain morphology– particularly in subcortical regions such as the striatum, amygdala, and hippocampus. These regions have a high prevalence of pubertal (androgen and estrogen) hormone receptors, with changes in hormone receptor density identified during puberty [1–3]. Further, pubertal hormones have been

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shown to alter the neuronal structure of these regions, including the density of dendritic spines [4–6]. However, there is a need for comprehensive and longitudinal investigation of the role of puberty and associated hormones in subcortical brain development in humans, with important implications for understanding emotional and behavioral development during this period.

Puberty begins with adrenarche, when activation of the hypothalamic-pituitary-adrenal (HPA) axis produces androgens (e.g., dehydroepiandrosterone [DHEA] and its sulphates) responsible for pubic hair growth and skin changes [7]. This is followed by gonadarche, when activation of the hypothalamic-pituitary-gonadal (HPG) axis results in the ovaries and testes producing sex hormones (estradiol and testosterone in females and males, respectively) responsible for reproductive maturity and secondary sex characteristics [8]. While puberty is commonly measured via the five "Tanner" stages of physical development [9], hormone changes begin years prior to observable physical changes [10,11] and there is variability in the correspondence between hormonal and physical changes [12]. Thus, examination of pubertal hormone concentrations may provide unique insight into the underlying endocrine processes that contribute to subcortical brain development.

Neuroimaging research has primarily examined associations between pubertal stage (i.e., physical maturation) and subcortical structures. Some have identified increases in the amygdala and hippocampus volume with pubertal stage [13,14] and decelerating increases in longitudinal trajectories [15], while others have found null associations [16–18] or sex differences [15,16,19–22]. Within the basal ganglia, longitudinal studies have identified increases in volume of the caudate and putamen at earlier stages of puberty, followed by reductions at later stages [15,16]. Others have also noted sex differences in the relationship between pubertal stage and the nucleus accumbens and pallidum [22]. Thus, consistent findings of subcortical changes with physical maturation are limited, but some studies suggest nonlinear associations and sex differences.

Few studies have examined associations between pubertal hormones and subcortical structures, and even fewer have examined adrenarche, but preliminary findings indicate positive correlations with pituitary [23] and hippocampal volumes [24]. Most studies have focused on testosterone, which has both adrenal and gonadal origins in males, but predominantly adrenal origins in females. Early cross-sectional studies identified varied associations between testosterone and subcortical volumes (i.e., positive, negative and null effects; [14,17,25,26]), although a recent longitudinal investigation found that testosterone explained more variance in a number of regions than pubertal stage [18]. Another longitudinal study found nonlinear associations between testosterone levels and the amygdala (in males) and striatum, characterized by volumetric increases at lower levels of testosterone and decreases at higher levels [16]. Finally, few studies have examined the role of the female gonadal hormone, estradiol. The only longitudinal study identified positive associations between estradiol levels and amygdala volume in females [16], but most cross-sectional work has found null effects across the subcortex [14,17,26,27]. Thus, our understanding of hormone-related changes in the human subcortex remains limited. Inconsistent findings may relate to a range of methodological limitations including small cross-sectional samples with limited power to detect nonlinear structural changes that are characteristic of the subcortex [28].

It is also important to consider inter-individual differences in pubertal maturation such as tempo – the rate of progression through pubertal stages, or the rate of hormonal increases [29]. As faster tempo predicts internalizing and externalizing symptoms [29–31], examination of pubertal tempo may provide novel insight into the development of brain regions that are implicated in mental health. We have shown that faster Tanner stage tempo predicts accelerated patterns of normative cortical thinning [32]. Further, Barch et al. [33] showed that faster testosterone increases were related to accelerated hippocampal development between 7 and 20 years of age. While rate of DHEA change was not related to subcortical development, the authors suggest that the age distribution of their sample may have lacked power to capture earlier adrenarcheal processes.

1.1. Current study

The current study addressed these limitations by comprehensively characterizing both group-level trajectories of subcortical development related to puberty and its hormones, as well as individual differences in these developmental processes. We used data from two longitudinal community-based cohort studies, which assessed children up to three times between late childhood and mid-adolescence (8.5–14.5 years of age). While our prior work on this sample has characterized the association between Tanner stage and cortical structure [32], the focus of this investigation was to better understand the role of specific pubertal hormones, and based on animal research, we expected these effects to be prominent in the subcortex. We interrogated DHEA and testosterone that represent adrenarcheal hormones regulated by the HPA-axis. Testosterone also has HPG origins in males during later gonadarche, but the younger age range of our sample means we were more powered to capture adrenarcheal processes. We also examined estradiol in females, which is a gonadarcheal hormone regulated by the HPA-axis. Additionally, we present the association with Tanner stage to provide a more complete picture of subcortical development during puberty.

The first two aims were to examine changes in subcortical structures in relation to pubertal stage and hormone levels. Given inconsistencies in the literature, we primarily based our hypotheses on prior longitudinal (as opposed to cross-sectional) studies that were better powered to identify developmental processes. First, we hypothesized that amygdala and hippocampal volumes would exhibit nonlinear (decelerating) increases with pubertal stage, while basal ganglia structures may exhibit nonlinear "peaks" (i.e., increases followed by reductions). Second, we hypothesized that DHEA would be positively associated with subcortical volumes, testosterone would be nonlinearly associated with structures (i.e., increases at lower concentrations and decreases at higher concentrations), and estradiol would be positively associated with amygdala volumes. We also explored potential sex differences (except for estradiol, which was only investigated in females), given that prior literature has identified sexual dimorphism in subcortical development [18,28,34]. The third aim was to examine whether pubertal tempo (i.e., rates of change in Tanner stage and hormone levels) were related to subcortical development within each sex, hypothesizing that faster pubertal tempo would relate to greater increases in the amygdala and hippocampus and earlier "peaks" in the basal ganglia.

2. Material and methods

2.1. Participants

Participants were recruited into one of two longitudinal cohorts based in Melbourne, Australia: i) Neuroimaging of the Children's Attention Project (NICAP), and ii) imaging brain development in the Childhood to Adolescence Transition Study (iCATS). NICAP participants were recruited as typically developing controls into a community-based study of children with and without ADHD. iCATS participants were recruited based on adrenal hormone levels (dehydroepiandrosterone and testosterone), such that those with the lowest or highest tertiles of these hormones, as measured approximately six months earlier in a larger parent project (CATS), were recruited. Note that while the iCATS recruitment procedure was designed to maximize variance in hormone levels in the sample, children that later participated in iCATS brain imaging had a distribution of hormone levels similar to children in NICAP (i.e., positively skewed). Further details on the NICAP and iCATS cohorts are presented in Silk et al. [35] and Simmons et al., [36], respectively. For both cohorts, written informed consent was obtained from the parent/guardian of all participants, and ethics approval was

granted by The Royal Children's Hospital Human Research Ethics Committee, Melbourne (NICAP #34071; iCATS #32171). Protocols were also ratified by the Human Research Ethics Committees of Deakin University (NICAP #2016-394) and The University of Melbourne (iCATS #1238745).

The NICAP cohort underwent up to 3 assessments between the ages of 9.5 and 14.5 years, with two approximately 18-month intervals (M = 1.432, SD = 0.222, 1.021–2.330 years) between assessments. The iCATS cohort underwent 2 assessments between the ages of 8.5 and 13.5 years, with one approximately 36-month interval (M = 2.763, SD = 0.243, 2.158–3.344 years) between assessments. At baseline (wave 1) the two cohorts did not differ in sex (note that both cohorts only examined biological sex, not gender identity; $\chi^2 = 1.342$, df = 1, p = 0.247), pubertal stage (Mean: NICAP = 1.316, iCATS = 1.282, $t_{(161)} = -0.431$, p = 0.666), or intelligence (based on Wechsler Abbreviated Scale of Intelligence – Matrix Reasoning T-score; Mean: NICAP = 52.607, iCATS = 54.096, $t_{(179)} = 1.225$, p = 0.222). However, the iCATS sample was significantly younger than the NICAP sample at baseline (Mean: NICAP = 10.425, iCATS = 9.556, $t_{(157)} = -14.928$, $p < 0.001$) and had higher socioeconomic status (based on Socio-Economic Indexes for Areas – Index of Relative Socio-economic Advantage and Disadvantage [based on Australian Census data]; Mean: NICAP = 1018.326, iCATS = 1056.175, $t_{(198)} = 4.887$, $p < 0.001$). In total, 192 participants (96 males, 90 NICAP) were included, with a total of 366 scans (186 males, 207 NICAP). Exclusion criteria for the current analyses included MRI contraindications, developmental disability, history of a neurological or serious medical disorder (e.g., diabetes, kidney disease), and concurrent use of psychotropic medications. Further sample characteristics are outlined in detail in Vijayakumar et al. [32] and summarized in the Supplement.

2.2. MRI acquisition & processing

Structural MRI scans were acquired across all waves in both cohorts. Participants were given information on MRI (including a video) prior to their assessment, in order to familiarize them with the procedure and minimize anxiety as much as possible. Participants completed a mock MRI scan before their actual scan at wave 1 (repeated at subsequent waves if the participant wished or the researcher deemed it appropriate). Neuroimaging data for both cohorts were acquired at a single site, on a 3 Tesla Siemens scanner (Siemens, Erlangen, Germany) at the Murdoch Children's Research Institute in Melbourne, Australia. Both waves of iCATS, and waves 1 and 2 of NICAP, were collected on a TIM Trio scanner. The final wave of NICAP was collected after an upgrade to a MAGNETOM Prisma scanner, which has been accounted for within statistical modelling. Further, refer to the Supplement for analyses showing there were no significant effects of scanner upgrade on subcortical volume (Table S3).

Participants lay supine in a 32-channel head coil during the MRI scan. Structural T1-weighted images were acquired as follows: *NICAP* – MEMPRAGE with repetition time = 2530 ms, echo time = 1.77, 3.51, 5.32, 7.2 ms, flip angle = 7°, field of view = 230 mm^2, resulting in 176 contiguous slices with voxel dimensions 0.9 mm^3; *iCATS* – MPRAGE with repetition time = 1900 ms, echo time = 2.24 ms, flip angle = 9°, field of view = 230 mm^2, resulting in 176 contiguous slices with voxel dimensions 0.9 mm^3.

T1-weighted images were processed through FreeSurfer 6.0, a freely available image analysis suite for cortical reconstruction and volumetric segmentation (http://surfer.nmr.mgh.harvard.edu/). Specifically, images were processed with the submillimeter reconstruction [37] and the longitudinal stream that creates an unbiased within-subject template space from all available data using robust, inverse consistent registration. The template is used as an estimate to initialize segmentation processes for each time point, providing common information regarding anatomical structures, and has been found to significantly increase reliability and statistical power [38,39]. The quality of i) raw images and ii) (longitudinal) cortical reconstructions was visually inspected and rated for all scans. Raw images were rated on a 4-point scale for "ringing" (1: no ringing; 2: slight ringing restricted to a small cortical area; 3: more ringing extending into white matter and/or covering more brain regions; 4: extensive ringing) and "blurriness" (1: sharply defined images; 2: slight blurriness; 3: or considerable blurriness; 4: blurring throughout). Ratings of "3" and "4" on either scale were excluded. Processed images were rated on a 3-point scale on the accuracy of the white and pial surfaces (1: near perfect reconstruction; 2: minor reconstruction issues limited to small areas of the brain; 3: poor reconstruction with consistent under-estimation of white matter or extensive areas of CSF included as grey matter). Ratings of "3" were excluded. Images were also processed through MRIQC (v0.14.2) to supplement the visual inspection [40]. This quality control process identified a total of 37 scans from 34 participants (21 male) that were excluded (see Ref. [32] for further detail on quality control). No manual edits were made to the remaining (included) data. Subcortical structures were automatically segmented and volumes were extracted and used in subsequent analyses, including the amygdala, hippocampus, caudate, putamen, pallidum and accumbens. We additionally examined the thalamus for a complete analysis of subcortical structures segmented in FreeSurfer, but did not have specific hypotheses for this structure.

2.3. Puberty

Pubertal stage was measured across all waves, in both cohorts, using the parent-report Sexual Maturity Status [41]. This survey consists of a series of stylized line drawings of girl's/boy's bodies at differing stages of pubertal development. Parents with a female child looked at drawings of five stages of breast development, and five stages of hip and pubic hair development, and chose the images that most accurately represented their daughter's development. Parents with a male child completed the same task, but with a single series of images of the five stages of male genital and pubic hair development. While these images directly correspond to the five Tanner stages of pubertal development and have been shown to have good reliability with physician ratings, they are considered to reflect "perceived pubertal stage" as opposed to objective measurements of Tanner Stage by physicians [42]. For females, the higher score for breast or pubic hair development images was used if there was a discrepancy (N = 69, 38%), as done previously [23,43]. Additionally, for females that only had data for either breast or pubic hair (N = 9, 5%), the single available score was used to measure Tanner Stage. Correlations between parent report of Tanner stage and physical exam range from 0.75 to 0.87, suggesting good validity [44], although we note that physical examination is not necessarily a gold standard measure of puberty. Further, correlations are stronger for children/adolescents at lower Tanner stages [45], suggesting that validity may be higher in the current sample. Moreover, parent- and self-report relate similarly to hormone concentrations [46].

2.4. Hormones

Hormones were assayed in saliva samples across both waves in iCATS and two out of three waves in NICAP (waves 1 and 3). Thus, a subset of 188 participants (94 males, 86 NICAP) contributed to DHEA and testosterone analyses, with a total of 291 scans (147 males, 132 NICAP). Further, estradiol was assayed in a cross-sectional sample of 42 females from the iCATS cohort alone.

iCATS: A waking saliva sample was collected from participants on the day of the MRI scan. Time of collection varied across participants (5.40 a.m.–12.55 p.m.), but this was not associated with testosterone concentrations (B = 0.001, SE = 0.001, T = 0.59, p = 0.56) or DHEA concentrations (B = 0.001, SE = 0.001, T = −0.06, p = 0.96). *NICAP:* A saliva sample was collected during the assessment, prior to the MRI scan. Time of collection varied across participants (8.30am–12.30pm), which was trending towards a significant association for testosterone

concentrations (B = 0.002, SE = 0.001, T = 1.904, p = 0.059) but not DHEA concentrations (B = 0.002, SE = 0.002, T = 0.995, p = 0.322). Time of collection was regressed out of hormone concentrations (within each cohort) prior to analyses (i.e., we used residual hormone values). For both cohorts, approximately 2.5 ml of saliva was collected per sample, via passive drool into a test tube. Samples were frozen at −30 °C, and prior to analysis, defrosted and centrifuged, with the supernatant assayed in duplicate for levels of DHEA and testosterone using Salimetrics enzyme-linked immunosorbent assay (ELISA) kits. DHEA and testosterone were log-transformed given positive skew. Additionally, estradiol was assayed in wave 2 of iCATS alone. Intra- and inter-assay coefficients of variation for each cohort are presented in Table S4. The sensitivity and range for each hormone are as follows: DHEA = 5 pg/ml; 10.2–1000 pg/ml; testosterone = 1 pg/ml; 6.1–600 pg/ml; estradiol = 0.1 pg/ml; 1–32 pg/ml. Refer to Fig. 1 for an illustration of hormone levels across age and Tanner Stage.

2.5. Statistical analyses

First, imputation was undertaken using "mice" [47] in R to deal with missing Tanner stage and hormones. Out of the full 366 observations across all waves, there were 32 data points missing for Tanner Stage (9%, 20 males). Out of the 291 observations across the first and final waves when hormones were examined, 19 data points were missing for testosterone (7%, 10 males) and 20 were missing for DHEA (7%, 10 males). Imputation was conducted on a long-format data set, and variables included sex, age, Tanner stage, testosterone and DHEA. A proportional odds model was used to impute the ordinal Tanner stage variable and predictive mean matching was used for continuous hormone variables. A total of 30 imputations were run, and the average mode and mean values across these imputations was used as the final Tanner stage and hormone concentrations, respectively.

Aims 1 and 2. Generalized additive mixed models (GAMMs) were used to examine longitudinal development of subcortical volumes in relation to Tanner stage (TS) and hormones (testosterone and DHEA). GAMMs were chosen as they enable flexible modelling of the relationship between independent and dependent variables, without specification of a particular form of association (i.e., linear vs nonlinear). As an exploratory aim, sex differences in puberty-related subcortical development were also investigated. The following models were examined using the "gam" function within the "mgcv" package [48] in R:

Puberty-related subcortical development: gam(volume ~ sex + s(study, bs = "re") + s(scanner, bs = "re") + s(id, bs = "re") + s(puberty, bs = "cr", k = 4), method = "REML", select = TRUE)

Sex differences in puberty-related nonlinear subcortical development: gam(volume ~ sex + s(study, bs = "re") + s(scanner, bs = "re") + s(id, bs = "re") + s(puberty, bs = "cr", k = 4) + s(puberty, by = sex, bs = "cr", k = 4), method = "REML", select = TRUE)

Models were repeated with TS, testosterone and DHEA as the given index of "puberty", and each subcortical volume as the dependent variable. The s(puberty, bs = "cr", k = 4) term represents developmental *smooth* terms using a penalized cubic regression spline and a basis function of 4 (chosen given the constrained age span that was investigated). The "s(study, bs = "re")", "s(scanner, bs = "re")", and "s(id, bs = "re")" terms represent the random intercepts for study (iCATS/NICAP), scanner (pre-/post-upgrade), and each individual, respectively. Sex was an ordered factor, in order to examine the developmental (trajectory) difference between males and females. As estradiol levels were only available from the second wave of the iCATS cohort, and analyses were only conducted in females, the following cross-sectional model was examined: gam(volume ~ s(estradiol, bs = "cr", k = 4), method = "REML", select = TRUE). All models were also re-analysed while controlling for age-related development, by including a *smooth* term of age (i.e., s(age, bs = "cr", k = 4)), thus controlling for potential linear and nonlinear effects of age.

Models were examined with restricted maximum likelihood (method = "REML") estimation. Model selection was undertaken using a double penalty approach (select = TRUE), which has a default penalty applied to each smooth term to determine non-linearity (or shrinkage to linearity) and a second penalty that also allows a linear term to be shrunk to null. Together, both penalties can result in a smooth term being removed from the model (i.e., effective degrees of freedom tends towards 0). This approach to model selection has been argued to be superior to iterative/step-wise procedures that are unduly influenced by the order of steps [49]. Significance of relevant terms (i.e. s(puberty, bs = "cr", k = 4)) was determined following correction for multiple comparisons using False Discovery Rate 0.05, to account for the 14 subcortical regions examined. Finally, we also conducted post-hoc analyses that examined puberty-related subcortical development within each sex (i.e., gam(volume ~ s(study, bs = "re") + s(scanner, bs = "re")

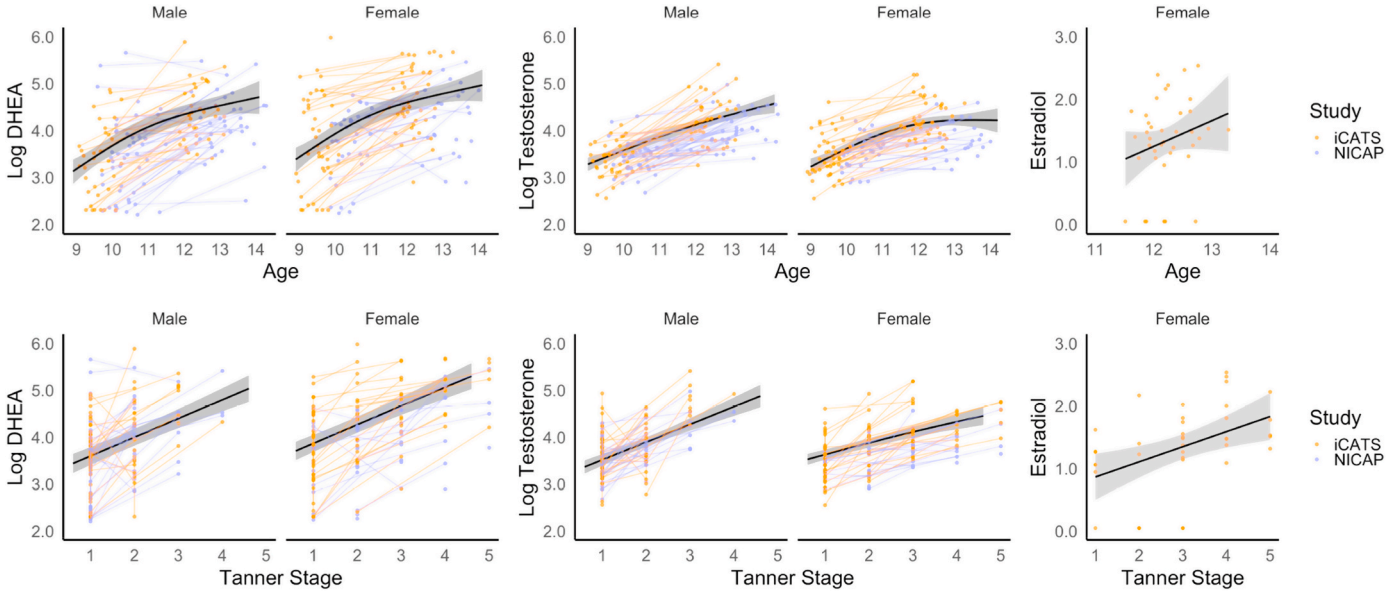

Fig. 1. Associations between hormones, age and Tanner Stage, illustrated for each sex and color-coded by study. Refer to the supplement for further details on the statistical modelling of hormone changes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

+ s(id, bs = "re") + s(puberty, bs = "cr", k = 4), method = "REML", select = TRUE).

Aim 3. Calculating hormonal tempo: A residual change score was used to calculate DHEA and testosterone tempo (i.e., residual scores from the following regression: hormone at final wave ~ hormone at baseline + age at baseline + age interval + cohort). Change scores were calculated between hormone concentrations at the first and last waves of each cohort (wave 3 and 1 of NICAP, and waves 2 and 1 of iCATS). *Calculating Tanner stage tempo:* As there were up to three waves of pubertal stage observations, residual scores from linear regression cannot model all available data. Thus, TS tempo was calculated using linear mixed models of TS (in Stata 15), consistent with methods used in our prior analyses of tempo [32]. We predicted TS from age and age^2, with random effect estimates for id and age (i.e., TS ~ age + age^2, random effect = [1 + age | id]). Random age slopes were extracted for each individual as an index of pubertal tempo as they reflect individual differences in the linear age-slope relative to the group. Random intercepts were also extracted as an index of TS at baseline. All tempo models were estimated for each sex separately, as females tend to progress through puberty 1 or 2 years prior to males [42]. Higher tempo values suggest that individuals are progressing through the Tanner stages faster, or exhibiting greater increases in hormones, than their peers over time (i.e., steeper positive trajectory than the group-level "fixed-effect" trajectory). Further detail on the calculation of pubertal tempo is presented in Vijayakumar et al. [32].

Next, TS/hormonal tempo was used to examine individual differences in subcortical development. Within each sex, we examined whether changes in subcortical volume over time differed as a function of tempo using the following model (which controlled for baseline levels of TS/hormones):

Tempo-related variability in subcortical development: gam(volume ~ s(study, bs = "re") + s(scanner, bs = "re") + s(id, bs = "re") + tempo + s(age, bs = "cr", k = 4) + s(age, by = tempo, bs = "cr", k = 4) + baseline puberty + s(age, by = baseline puberty, bs = "cr", k = 4), method = "REML", select = TRUE)

The s(age, by = tempo, bs = "cr", k = 4) term represents a linear interaction between the *smooth* age term and tempo, and informs us whether individual differences in TS/hormonal tempo were associated with different rates of subcortical development over age/time. We checked whether the s(age, by = tempo, bs = "cr", k = 4) term was significant following correction for multiple comparisons using False Discovery Rate 0.05, to account for the 14 subcortical regions examined. "Baseline puberty" values were random intercepts for TS, and measurements at first wave for hormones. We did not control for random intercept in TS tempo models in males as there was almost no variance in the random intercept (SD = 2.04 e-26).

Hormonal tempo analyses were conducted on all participants who contributed data from both baseline and final-wave assessments (thus permitting calculation of residual change scores). These analyses were comprised of 57 males (32 NICAP) with 135 observations (85 NICAP) and 51 females (21 NICAP) with 121 observations (61 NICAP). Pubertal tempo analyses were conducted on all participants who contributed to at least one wave (as all available data was used to calculate random slopes). All but 1 female and 1 male contributed to this analysis: 95 males (49 NICAP) with 185 observations (113 NICAP) and 95 females (39 NICAP) with 178 observations (91 NICAP).

2.5.1. Sensitivity analyses

A series of sensitivity analyses were conducted. First, we included body-mass index (BMI) and SES as additional covariates of non-interest to models. SES was based on the Socio-Economic Indexes for Areas – Index of Relative Socio-economic Advantage and Disadvantage from Australian Census data. BMI was calculated from height measurements (to the nearest 0.1 cm) and weight measurements in clothing (to the nearest .1 kg). Second, we re-ran analyses with the exclusion of 11 participants on steroid medications in the year prior to assessment (males = 6, NICAP = 0). Third, we re-ran analyses with the exclusion of 5 participants who exhibited reductions in Tanner stage over time (males = 5, NICAP = 2). We did not run additional analyses that controlled for whole brain volume/intracranial volume, as these continue to develop during adolescence [66] and subcortical regions show differential scaling when corrected for whole brain size during this period [50]. Further, doing so also violates the statistical assumption of equal variances between males and females [34].

3. Results

3.1. Tanner stage and subcortical development

Models revealed significant developmental trajectories across most of the subcortex, including positive linear associations between Tanner stage (i.e., parent perceived physical maturation) and the bilateral hippocampus and pallidum, and right amygdala and left caudate (see Fig. 2). Nonlinear associations were identified with the bilateral putamen and left thalamus, although exploratory analyses of sex differences revealed that these were driven by females. Post-hoc analyses within each sex further highlighted no association in males. Refer to Tables S5 and S6 for summaries of models that did not control for age. When controlling for age, the only findings that remained significant were sex differences in the developmental trajectories of the bilateral putamen (see Tables S7 and S8). Given the greater sampling of females at later Tanner stages relative to males, sex differences were re-examined when limiting both sexes to Tanner stages 1–3. The results remained consistent, although the shape of nonlinear trajectories in females slightly differed (see Fig. S3). Also refer to the Supplement for further post-hoc exploration of adrenal- and gonadal-driven physical changes separately (Fig. S6).

3.2. Hormones and subcortical development

DHEA was significantly related to volumes of the bilateral amygdala, hippocampus, and pallidum. Effects for the left amygdala and left hippocampus were characterized by nonlinear associations, while all other regions exhibited a positive linear relationship. When controlling for age, only associations for the left amygdala remained significant. Furthermore, there were no sex differences in these associations, both with and without controlling for age. Results are illustrated in Fig. 3 and presented in Tables S5–S8, including post-hoc analyses conducted within each sex.

Testosterone was significantly related to volumes of the bilateral amygdala, hippocampus, and pallidum, as well as the right caudate and left accumbens. Effects were primarily characterized by a positive linear relationship between testosterone and subcortical volumes, apart from negative associations for the left accumbens and nonlinear associations for the left hippocampus and right caudate. Results are illustrated in Fig. 3 and presented in Tables S5 and S6, including post-hoc analyses conducted within each sex. When controlling for age, associations between testosterone and the left amygdala remained significant (see Tables S7 and S8). Effects in the left accumbens (without controlling age) and right caudate (controlling for age) were further characterized by sex differences. Specifically, in females, testosterone was associated with linear decreases in the accumbens and non-linear "peaks" in the caudate, whilst in males there were no associations (see Fig. S5).

Finally, there were no significant associations between estradiol levels and subcortical volumes in females (see Table S9).

3.3. Pubertal tempo and subcortical development

Interactions between Tanner stage tempo (i.e., random slopes) and age, and hormonal tempo (i.e., residual change) and age, in relation to

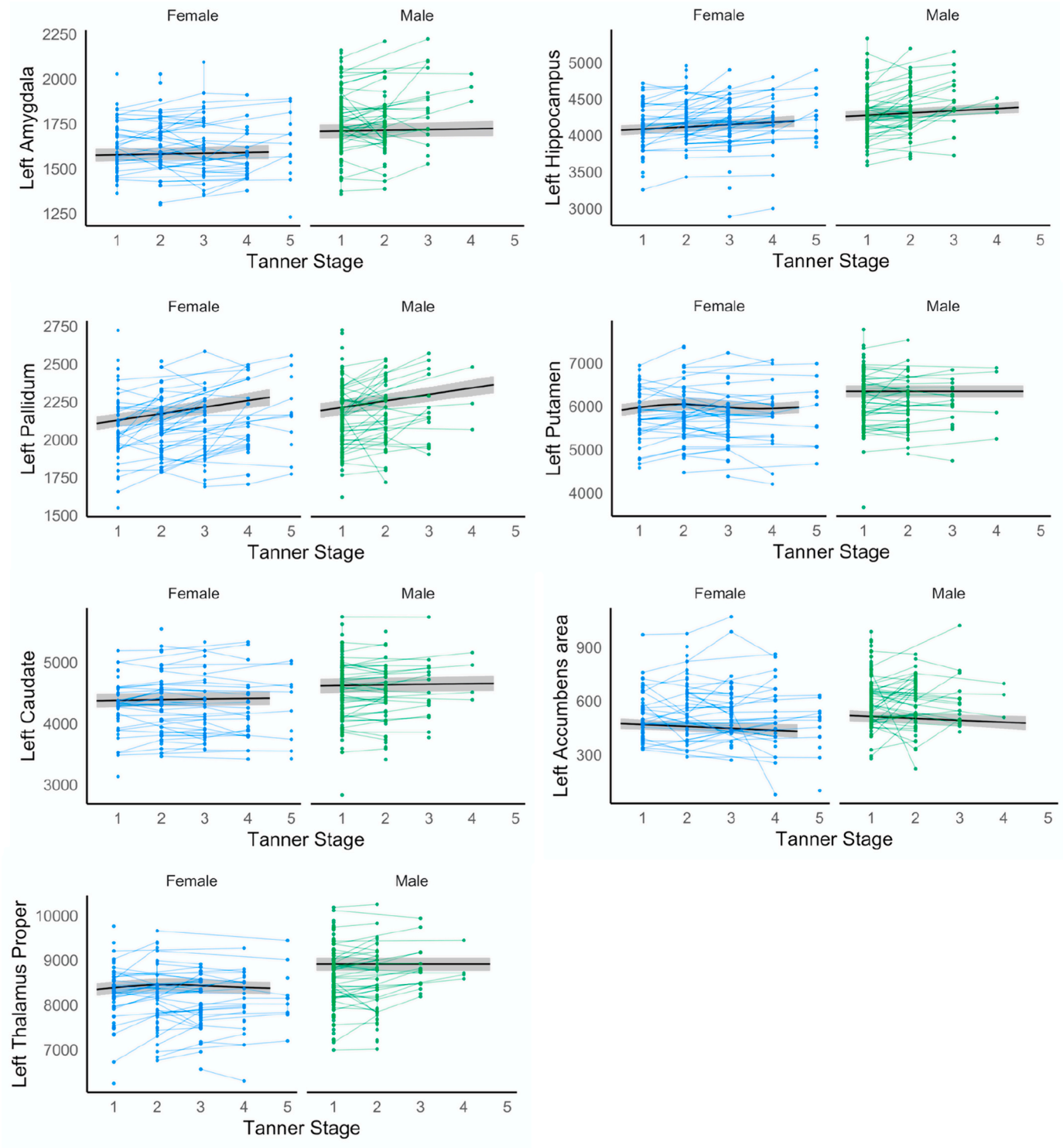

Fig. 2. Tanner stage-related changes in the subcortex in the left hemisphere (without controlling for age). See Fig. S2 for effects in the right hemisphere, which were largely consistent to the left hemisphere.

subcortical volumes were examined within each sex. We found significant interactions between testosterone tempo and age predicting right hippocampal volume in males. Specifically, those who exhibited faster hormonal tempo (i.e., greater rates of increase) also exhibited greater increases in volume over time (see Fig. 4 and Table S10). No effects reached significance following FDR correction in females (see Table S11).

3.4. Sensitivity analyses

Sensitivity analyses were conducted *i)* by controlling for BMI and SES, *ii)* with the exclusion of participants on steroid medications over the year prior to the assessment, and *iii)* with the exclusion of participants who exhibited reductions in Tanner stage over time. Across these analyses, the majority of results, and the overall pattern of results, remained consistent (see Tables S5–S11).

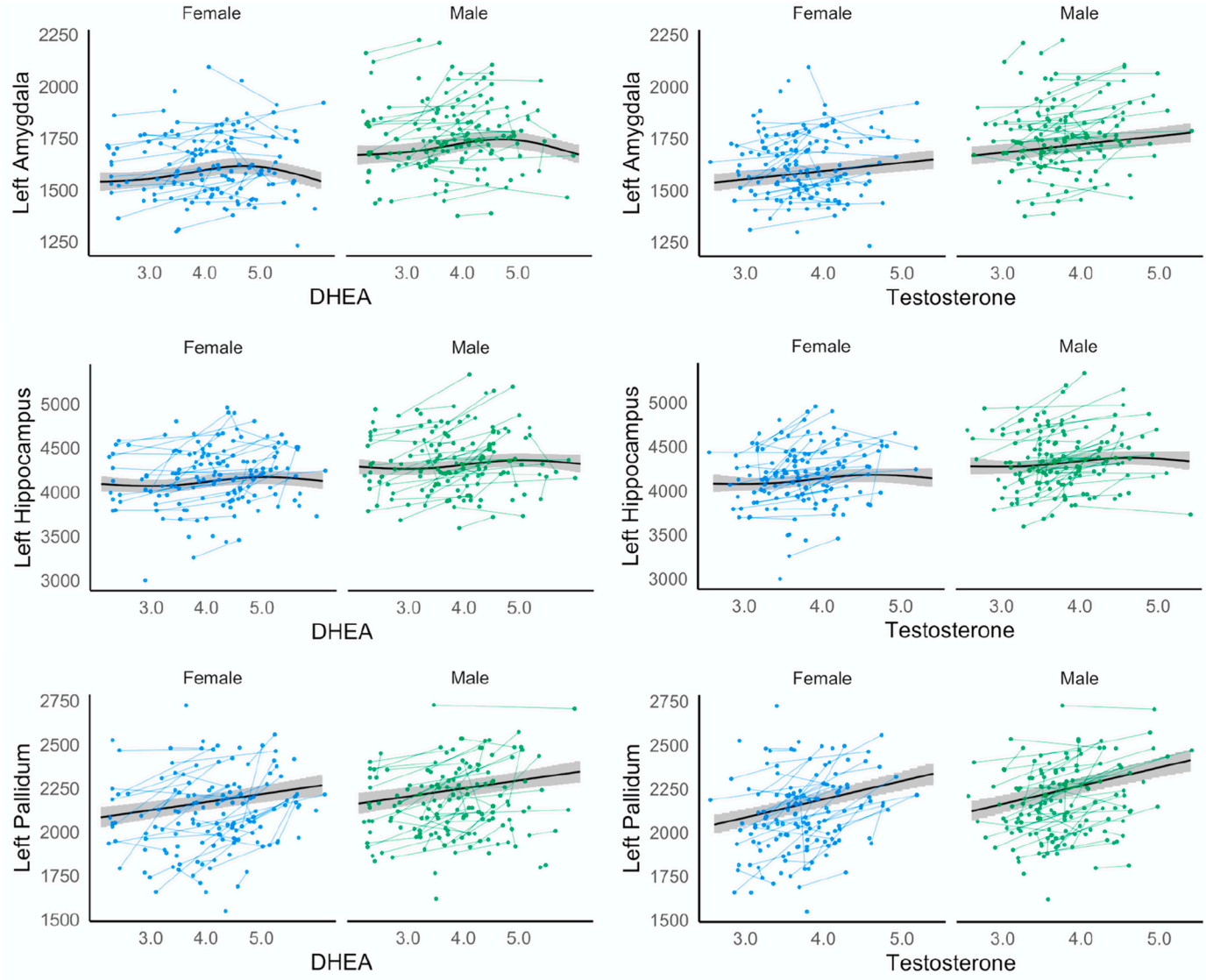

Fig. 3. Hormone-related changes in the subcortex in the left hemisphere (without controlling for age). See Fig. S4 for effects in the right hemisphere, which were characterized by positive linear associations.

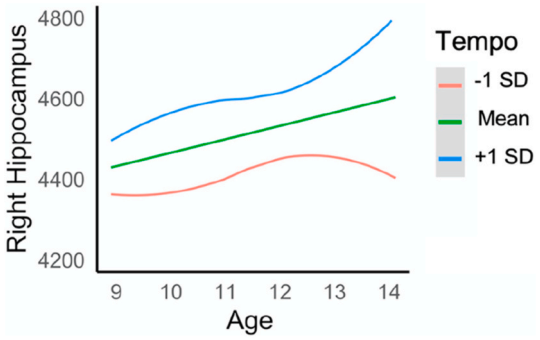

Fig. 4. Variability in hippocampal development as a function of testosterone tempo in males. + 1 SD represents those males with relatively faster testosterone tempo.

4. Discussion

The current study investigated puberty-related changes in subcortical structures using a longitudinal sample of 8–14 year-olds. Findings demonstrated that development of the amygdala, hippocampus and pallidum was related to Tanner stage and testosterone, and for the first time we show that similar associations were present for DHEA. When controlling for age, hormones but not Tanner stage predicted amygdala development. We also found that variability in testosterone tempo was related to right hippocampal development in males.

4.1. Tanner stage and subcortical development

As hypothesized, we found that changes in the bilateral hippocampus and right amygdala were related to Tanner stage (i.e., parent perceived physical maturation). However, these changes were linear in trajectory between 8 and 14 years of age. Inconsistencies in the exact shape of volumetric increases are most likely related to the wider and older age range, and thus greater density of late Tanner stages, in prior literature (e.g., 7–20 year-olds; [15]. Indeed, other longitudinal research of a similar age range (10–16 year-olds) identified linear increases in the amygdala with pubertal stage (although in males alone; [16]). Thus, puberty-related growth in the amygdala and hippocampus may only decelerate around mid-adolescence. There was also inconsistent support for the hypothesis of Tanner stage being related to nonlinear “peaks” in the basal ganglia; we identified the expected nonlinear trajectories in the

bilateral putamen, but linear increases in the bilateral pallidum and left caudate. While Goddings et al. [15] also found nonlinear change in the putamen, they comparatively found linear decreases in the pallidum. Wierenga et al. [18,34] identified nonlinear change in the pallidum over early to late adolescence, but consistent with our findings, it appears that linear increases may be present prior to mid-adolescence. Taken together, our findings highlight distinct developmental trajectories of subcortical structures during puberty, and comparisons with prior literature suggest these patterns may be highly influenced by the age range and distribution of Tanner stage in a given sample. None of our Tanner stage results remained significant when controlling for age, suggesting that findings were not driven by individual differences in pubertal stage at a given age, but rather reflect normative progression through Tanner stages across the age range.

Interestingly, our exploratory analyses of sex differences provided more support for the expected Tanner stage-related nonlinear "peaks" in the basal ganglia, which were identified in the bilateral putamen in females alone (both with and without covarying for age). Similar effects were also present in the left thalamus without covarying for age. Nonlinear trajectories in females were characterised by an early "peak" followed by stabilization during late Tanner stages. In order to determine whether these sex differences were driven by the uneven sampling of late stages in the two sexes, supplemental analyses were restricted to Tanner stages 1–3. While sex differences remained significant, the trajectories in females were characterized by "peaks" without the stabilization that occurs in late stages. As post-hoc exploration, we examined subcortical development in relation to adrenal- and gonadal-driven physical changes in females (specifically pubic hair and breasts, respectively), as our measure of Tanner stage allows for interrogation of each of these features separately in females alone. Although we identified largely similar effects across both features, findings need to be replicated in samples with more even distribution across the different stages of physical development. Thus, there remains a need for future research that covers early and late stages of physical development in both males and females, as well as measures that distinguish adrenal and gonadal maturation in each sex.

4.2. Hormones and subcortical development

The current study presents the first investigation of DHEA-related changes across the subcortex. Findings revealed significant associations between DHEA and volumes of the bilateral amygdala, hippocampus, and pallidum. Results supported our hypothesis of linear associations between hormone concentrations and subcortical volumes in most of these regions. There has been minimal research to date on the role of DHEA in human brain development. Our work has previously shown that DHEA levels also positively correlate with pituitary volumes in a similar age range [23] and hippocampal volumes during late childhood [24]. Consistently, animal research suggests that DHEA supports neurogenesis [51] and modulates neurotoxic processes in the hippocampus via anti-glucocorticoid and anti-oxidant processes [52,53]. Others have shown that DHEA levels influence structural and functional amygdala connectivity with the cortex in humans [54], and that such connectivity may support cognitive development [55]. Thus, findings highlight the importance of adrenarcheal processes, which are unique to humans and higher primates [56], for neurobiological maturation.

There was also partial support for our hypothesis regarding testosterone-related changes in subcortical volumes. We specifically identified testosterone-related increases in the bilateral amygdala, hippocampus and pallidum, and right caudate and left accumbens. However, the expected nonlinear changes were only present in the left hippocampus and right caudate, with most other structures exhibiting linear increases in volume with rising testosterone levels. Hypotheses of nonlinearity were based on prior longitudinal findings of age and testosterone interactions predicting amygdala and caudate changes, such that volumes increased at lower testosterone levels and decreased at higher levels [16]. Moreover, rodent research has shown that pubertal testosterone decreases the volume and neuronal number of the amygdala, although this is largely limited to anterior portions [57]. As with findings for pubertal stage, we speculate that we may have failed to identify potential apoptotic effects of testosterone because of lower concentration levels in our participants who clustered towards lower and middle Tanner stages. Interestingly, these same regions (i.e., amygdala, hippocampus, pallidum, caudate and accumbens) were implicated in Wierenga et al.'s [18,34] investigation of group-level associations between testosterone levels and subcortical volumes, highlighting converging evidence for regional specificity of the effects of testosterone in the subcortex.

For both DHEA and testosterone, associations in the left amygdala alone remained significant when controlling for age, although similar associations in the right amygdala did not survive correction for multiple comparisons. Overall, the prominence of effects in the amygdala (with and without controlling for age) and hippocampus (without controlling for age) are consistent with the prevalence of androgen and estrogen receptors in these regions [2,3]. Studies have also noted changes in sex hormone receptor density during puberty, including increased androgen receptors in the amygdala following the onset of puberty [1] and more specifically with rising testosterone levels [58]. While comparatively minimal research has been conducted on the pallidum, aromatose signalling has been identified in mice and suggests local synthesis of estrogen may mediate the effects of testosterone in this region [59]. Taken together, our findings suggest potential neurotrophic effects of testosterone across these limbic structures during early-to-mid pubertal stages, which may also be most reflective of the earlier phase of adrenarche. Furthermore, the consistency of effects across males and females (including post-hoc analyses conducted within each sex), and the similarity in testosterone levels in both sexes (see Fig. 1), also supports the hypothesis that these effects are driven by earlier adrenarcheal processes (before gonadal production dramatically increases testosterone in males relative to females).

We failed to identify any associations between estradiol concentrations and subcortical volumes in females. Prior cross-sectional research has also failed to identify any associations between estradiol levels and subcortical volumes [14,17,26,27], with the only significant (positive) association between estradiol and amygdala volumes reported in a longitudinal study [16]. However, rodent research indicates that estradiol treatment increases neuronal spine density in the amygdala and hippocampus in ovariectomized females [4,60]. Thus, the human literature may be under-powered to identify estradiol-subcortical associations, highlighting the need for further longitudinal investigation in large samples. Another important consideration for future research relates to the measurement of estradiol, as menstrual cyclicity of estradiol concentrations may introduce additional noise into brain-hormone associations. Future studies may also benefit from using assay methods that are more sensitive to low estradiol concentrations than ELISA, such as liquid chromatography tandem mass spectrometry [42].

4.3. Pubertal tempo and subcortical development

Beyond group-level changes, we found that individual differences in hormonal tempo were related to variability in subcortical development. Specifically, males with faster testosterone tempo (i.e., greater increases in testosterone levels) also exhibited greater increases in right hippocampal volume. This is consistent with the only prior study that identified similar specificity of effects to testosterone and the hippocampus (as compared to DHEA and/or any other subcortical structures; [33]). Our finding likely reflects an accelerated pattern of normative subcortical development in adolescent males who progress through puberty faster than their peers, which is consistent with our prior work showing that faster Tanner stage tempo is related to accelerated patterns of normative cortical thinning (also identified in males alone; [32]). The

hippocampus plays a critical role in emotional responding, decision making, and the formation of long-term memories [61]. Thus, findings may be suggestive of altered maturation of these affective and cognitive processes in individuals who progress through puberty faster than their peers. Indeed, Barch et al. [33] showed that faster testosterone tempo and steeper hippocampal development were related to *less* emotional dysregulation and depression during late adolescence. However, faster pubertal tempo (based on observable physical changes) has also been shown to predict greater internalizing and externalizing symptoms [29,62,63], suggesting potentially distinct effects of testosterone that are mediated via hippocampal development.

The specificity of effects to males does raise potential limitations with our operationalization of tempo, which was limited to linear rates of change. As we previously discussed [32], linear change may more appropriately represent the earlier stages of pubertal maturation within males in our sample. Comparatively, the more extended period of puberty that was captured in females may be better represented by nonlinear trajectories, as identified in prior literature [29]. Thus, future studies with additional time points are need for more nuanced characterizations of tempo, in order to improve our understanding of the pubertal determinants of brain development and functional outcomes.

4.4. Limitations

Despite the strengths of the current study (a longitudinal sample with flexible modelling strategy), findings need to be considered in the context of some limitations. As discussed above, findings are dependent on the age range and distribution of pubertal/hormonal maturation, and future investigation of a larger age range will help unpack inconsistencies with prior literature. Further, although we speculate that our sample was more suited to capture adrenarcheal development, we are unable to distinguish potential adrenal and gonadal effects in some of our indices of puberty (e.g., testosterone in males) across the sampled age range. Additional issues relating to measurement tools include the use of parent-reported perceived pubertal stage (94% were mothers), although as discussed above alternate self-reported and clinician-rated measures also have limitations [64]. Further, hormone concentrations were examined from a single saliva sample, which are susceptible to momentary influences (in particular, circadian rhythmicity and menstrual cyclicity). Thus, future research should incorporate additional measures of pubertal stage and multiple saliva samples to obtain "basal" estimates. Additionally, the NICAP cohort was assessed on an upgraded scanner at the last wave of assessments. However, as this upgrade was not confounded with pubertal development (see Figure S3 in Ref. [32], we were able to account for potential scanner differences in our statistical models. Finally, we were unable to control for race and ethnicity, and given well-established race/ethnic disparities in age of pubertal onset [12,65], this represents an important consideration for future research.

4.5. Conclusions

The current longitudinal study highlights unique patterns of puberty-related subcortical development during 8–14 years of age, including novel associations with DHEA. Specifically, both Tanner stage and hormones (DHEA, testosterone) appear to predict amygdala, hippocampus and pallidum development, including some effects that are independent to age-related changes. Furthermore, our findings support the only prior investigation on individual differences in puberty and subcortical development, showing that hormonal (testosterone) tempo is related to accelerated maturation of the hippocampus. Findings are particularly suggestive of the important role of the earlier pubertal phase of adrenarche in adolescent brain development.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2021.100074.

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