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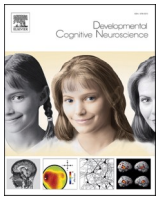

Fronto-amygdala resting state functional connectivity is associated with anxiety symptoms among adolescent girls more advanced in pubertal maturation

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ABSTRACT

Early adolescence, with the onset of puberty, is an important period when sex differences in anxiety emerge, with girls reporting significantly higher anxiety symptoms than boys. This study examined the role of puberty on fronto-amygdala functional connectivity and risk of anxiety symptoms in 70 girls (age 11–13) who completed a resting state fMRI scan, self-report measures of anxiety symptoms and pubertal status, and provided basal testosterone levels (64 girls). Resting state fMRI data were preprocessed using fMRIPrep and connectivity indices were extracted from ventromedial prefrontal cortex (vmPFC) and amygdala regions-of-interest. We tested moderated mediation models and hypothesized that vmPFC-amygdala would mediate the relation between three indices of puberty (testosterone and adrenarcheal/gonadarcheal development) and anxiety, with puberty moderating the relation between connectivity and anxiety. Results showed a significant moderation effect of testosterone and adrenarcheal development in the right amygdala and a rostral/dorsal area of the vmPFC and of gonadarcheal development in the left amygdala and a medial area of the vmPFC on anxiety symptoms. Simple slope analyses showed that vmPFC-amygdala connectivity was negatively associated with anxiety only in girls more advanced in puberty suggesting that sensitivity to the effects of puberty on fronto-amygdala function could contribute to risk for anxiety disorders among adolescent girls.

1. Introduction

As children enter adolescence, with the onset of puberty, pivotal sex differences emerge in the rise of anxiety symptoms, with girls exhibiting significant increases compared to boys (Beesdo-Baum and Knappe, 2012). This is notable because high levels of anxiety symptoms during this developmental period increase risk for anxiety disorders in adulthood (Pine et al., 1998), and other psychopathology such as depression and suicide (Barzilay et al.; Bittner et al., 2004; Brady and Kendall, 1992; Rice et al., 2017), especially in girls (Cole et al., 1998; Costello et al., 2003; Goodwin et al., 2004; Hale et al., 2009). However, the neurodevelopmental processes underlying the rise in anxiety symptoms in adolescent girls remain poorly understood. Some have proposed that changes in pubertal hormones play a role in this increased vulnerability to anxiety during early adolescence (Reardon et al., 2009) and that this could occur via influence of pubertal hormones on brain development

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(Ladouceur, 2012; Ladouceur et al., 2012; Pfeifer and Allen, 2021). One plausible neurodevelopmental pathway for such increased vulnerability in girls during this period is through the effects of puberty on fronto-amygdala function, which is implicated in emotion regulation and anxiety (Phillips et al., 2008; Silvers et al., 2017).

1.1. Fronto-amygdala circuitry and anxiety in youth

The amygdala is involved in emotional learning and facilitates attention to salient cues (Phelps and LeDoux, 2005). The vmPFC instantiates the regulation of emotions such as fear and anxiety (Etkin et al., 2011; Motzkin et al., 2015; Quirk and Beer, 2006; Urry et al., 2006), and includes the medial structures of the orbital frontal cortex (OFC) (BA 10, 11, 12) and rostral anterior cingulate cortex (rACC) (BA 25, lower 24). Recent neuroimaging work supports evidence from lesion and anatomical studies that functional and anatomical subregions of the vmPFC may exhibit distinct developmental trajectories (e.g., subcortical connections) (Chase et al., 2020). There are direct and reciprocal projections between these regions and the amygdala (Ghashghaei et al., 2007), with most of the amygdala-projecting fibers sent to the basolateral subnuclei (Little and Carter, 2013; Quirk et al., 2003). Evidence from rodent studies (Quirk and Beer, 2006; Selleck et al., 2018) as well as human lesion (Jenkins et al., 2018; Motzkin et al., 2015) and neuroimaging studies (Etkin et al., 2011; Ochsner and Gross, 2005; Phillips et al., 2008) support the inhibitory role of the vmPFC on amygdala activity, including the regulation of physiological signals of arousal (i.e., increased skin conductance and heart rate) (Banks et al., 2007; Etkin et al., 2011; Hariri et al., 2003; Perlman and Pelphrey, 2010; Phelps et al., 2004; Urry et al., 2006). The vmPFC and the amygdala also project to other regions implicated in emotion processing and regulation (e.g., bed nucleus of the stria terminalis (BNST), anterior insula, brainstem, hippocampus, lateral prefrontal cortex) (Myers-Schulz and Koenigs, 2012; Petrides, 2002) indicating that these regions are embedded within several neural networks.

The function and structure of the vmPFC and the amygdala change considerably during late childhood and adolescence (Gotgay et al., 2007; Guyer et al., 2016; Hare et al., 2008; Somerville et al., 2011; Swartz et al., 2014). They also undergo important age-related changes in functional connectivity (Casey et al., 2019; Gabard-Durnam et al., 2014; Gee et al., 2013; Wu et al., 2016) at rest (Gabard-Durnam et al., 2014; Jalbrzikowski et al., 2017) and during the processing of emotional information (Gee et al., 2013; Gold et al., 2020; Wu et al., 2016). In particular, when processing threat, vmPFC-amygdala functional connectivity indices shift from more positive (thought to index less inhibitory processes) in childhood to more negative (more inhibitory) in adolescence (Gee et al., 2013; Wu et al., 2016), with the point of change occurring in early adolescence (Gabard-Durnam et al., 2014). During rest, some have found age-related increases (Gabard-Durnam et al., 2014) whereas others (Jalbrzikowski et al., 2017) have reported decreases in vmPFC-amygdala functional connectivity; discrepancy in findings may be attributed to age range, study design (i.e., cross-sectional vs. longitudinal) and preprocessing pipelines (Bloom et al., 2022). Although resting state and task-related functional connectivity (e.g., psychophysiological interactions (PPI)) are generally correlated (Smith et al., 2009), there are important differences of interpretation between the two methods, which should be considered as complementary (O'Reilly et al., 2012). Nevertheless, given the regulatory role of the vmPFC on amygdala activity based on evidence from animal models (Quirk and Beer, 2006) and studies in adults (Banks et al., 2007), findings indicate that vmPFC-amygdala connectivity changes during adolescence and have been interpreted as reflecting increases in regulatory control of amygdala.

Altered regulatory control of vmPFC on the amygdala is thought to contribute to anxiety symptoms (Blackford and Pine, 2012; Madonna et al., 2019). Indeed, one of the most consistent findings in youth with anxiety, compared to youth without anxiety, is heightened amygdala activation (Blackford and Pine, 2012; Britton et al., 2010; Monk et al., 2008) and reduced fronto-amygdala connectivity to threat-related stimuli (Blackford and Pine, 2012; Monk et al., 2008; Strawn et al., 2012). Such patterns of fronto-amygdala functioning have also been found in at-risk youth (Abend et al., 2020; Sequeira et al., 2021) and linked to negative attentional biases and maladaptive emotion regulation strategies in the real-world (Price et al., 2016; Sequeira et al., 2021). Some have reported alterations in age-related vmPFC-amygdala resting state functional and structural connectivity associated with higher levels of anxiety and depressive symptoms in a longitudinal study with a community sample of 10–25 year-olds (Jalbrzikowski et al., 2017). Yet, the neurodevelopmental processes that contribute to vulnerability to anxiety symptoms during early adolescence, particularly in girls, remain unclear. We propose that puberty-related influences on fronto-amygdala connectivity could be one pathway by which girls become at increased risk for anxiety disorders during early adolescence.

1.2. Puberty, fronto-amygdala function, and anxiety

Puberty is characterized by the development of secondary sexual characteristics and attainment of reproductive capacity along two axes governed by separate mechanisms – adrenarcheal and gonadarcheal processes – that vary in age of onset and maturation rate (Dorn et al., 2006). Pubertal changes along the adrenarcheal axis begin around five to seven years of age and are characterized by the release of adrenal hormones (DHEA and testosterone) and physical changes such as changes in body odor, growth of pubic hair, and appearance of acne (Byrne et al., 2017). Changes along the gonadarcheal axis, which typically follow but overlap with those along the adrenarcheal axis, are associated with maturation of primary and secondary sexual characteristics (e.g., breast and genital development) driven by production of gonadal steroid hormones (testosterone and estradiol). Recent evidence indicates that pubertal hormones have organizing effects on the brain (Goddings et al., 2014; Herting and Sowell, 2017; Piekarski et al., 2017; Sisk and Zehr, 2005) and that pubertal timing could thus directly influence subsequent neurodevelopment.

There is growing evidence that individual variation in adrenarcheal and gonadarcheal processes are associated with symptoms of anxiety, with some evidence finding that earlier pubertal timing is associated with higher symptoms (Reardon et al., 2009) and that these associations may be mediated by hormonal effects on brain development (Juraska et al., 2013; Murray et al., 2016; Sisk and Foster, 2004; Sisk and Zehr, 2005). For instance, recent work suggests that early adrenarcheal timing may be linked to heightened anxiety (Barendse et al., 2018) via a more positive (or less mature) amygdala to inferior frontal cortex functional connectivity when viewing fearful (vs. calm) faces (Barendse et al., 2020). Indeed, given the high density of androgen and estrogen receptors in medial temporal regions (Sarkey et al., 2008; Simerly et al., 1990) (e.g., amygdala, prefrontal cortex) (Pfaff and Keiner, 1973; Simerly et al., 1990), the functioning of these neural regions may be directly influenced by changes in hormone levels with pubertal maturation. The modulating effects of testosterone on fronto-amygdala functional connectivity is evident from testosterone administration studies in adults demonstrating that higher levels of serum testosterone correlated positively with amygdala response to negative faces (Hermans et al., 2008; Van Wingen et al., 2010) and negatively with fronto-amygdala coupling (Van Wingen et al., 2010). In adolescents, increases in circulating testosterone over two years was associated with reductions in OFC-amygdala coupling to negative faces (Spielberg et al., 2015). Together, these findings suggest that earlier pubertal timing could lead to a rise in anxiety symptoms and that this relationship may be mediated by the effects of increases in pubertal hormones (i.e., testosterone) on vmPFC-amygdala resting state functional connectivity (i.e., reduced coupling). However, the rise in anxiety symptoms during this developmental period could also be linked to heightened sensitivity of the modulatory effects of pubertal hormones on the innervation of the

amygdala to the prefrontal cortex (Delevich et al., 2021; Piekarski et al., 2017). Individual variation in neurobehavioral sensitivity to changes in levels of pubertal hormones at the receptor level has been proposed as a potential contributing factor to hormone-related psychopathology in adulthood (e.g., postpartum or postmenopausal depression) (Rubinow and Schmidt, 2019). Thus, it is possible that some girls may be particularly sensitive to the modulatory effects of testosterone on fronto-amygdala function (Spielberg et al., 2015; Spielberg et al., 2019). In this case, levels of testosterone would play a moderating role in the association between fronto-amygdala functional connectivity and anxiety symptoms such that reduced connectivity would be associated with higher levels of symptoms in girls more advanced in pubertal maturation or who have higher levels of testosterone. Finally, higher anxiety symptoms associated with earlier pubertal timing could also be linked to alterations in girls' behaviors or those around them (e.g., peers, parents) given evidence that girls who self-report being more advanced in pubertal status reported higher levels of internalizing symptoms (Barendse et al., 2022). However, to what extent such behavioral alterations and social experiences in girls perceived as more pubertally mature is associated with vmPFC-amygdala connectivity and anxiety symptoms remains unclear.

1.3. Goal of the study and hypotheses

The current study tested the following primary hypotheses using a moderated-mediation model in a sample of early-adolescent girls varying in temperamental risk for anxiety disorders. The use of a moderated-mediation model afforded the possibility of testing both the mediating and moderating relationships between testosterone/pubertal development, vmPFC-amygdala functional connectivity, and anxiety symptoms. First, we hypothesized that levels of testosterone would moderate the relation between vmPFC-amygdala resting state functional connectivity and anxiety symptoms such that reduced vmPFC-amygdala functional connectivity would be associated with higher anxiety symptoms, particularly in girls with higher levels of testosterone. We also hypothesized that pubertal status would play a moderating role in the relation between vmPFC-amygdala connectivity and anxiety symptoms such that reduced functional connectivity would be associated with higher anxiety symptoms in girls more advanced in self-reported pubertal status, particularly in adrenarcheal processes related to the rise in testosterone in girls (Byrne et al., 2017). In both cases, we also hypothesized the vmPFC-amygdala connectivity would mediate the relations between testosterone/pubertal development and anxiety symptoms. Finally, our mediated moderation models included 'age' as a covariate to ensure that developmental effects were specific to puberty and not to effects of chronological age. Given the paucity of research on puberty-related influences on fronto-amygdala circuitry, we used a vmPFC parcellation to explore *which* subregions of the vmPFC would be related to testosterone/pubertal development and anxiety symptoms.

2. Methods

2.1. Participants

The data from a total of 70 participants was used in the reported analyses. Here we describe the process of how those 70 participants were derived from the larger sample.

The overall sample consisted of 129 early adolescent girls ages 11–13 who were recruited for participation in the study via online advertisements and flyers in the community. Recruitment of participants was based on parent's report of their child's sex assigned at birth; gender identity was not assessed at intake. The current study is part of a larger longitudinal study investigating how social threat sensitivity in early adolescence confers risk for social anxiety and depression in mid- to late-adolescence. Thus, recruitment was based on oversampling for shy/fearful temperament, which is a risk factor for social anxiety disorder into adolescence and adulthood (Chronis-Tuscano et al., 2009). Temperament was assessed using online screening form prior to participants' first visit using the Early Adolescent Temperament Questionnaire-Revised (EATQ-R; Ellis and Rothbart, 2001). The EATQ-R was designed to measure temperament traits in early adolescence (ages 9–15), with items specific to adolescent life experiences. To determine temperament status, participants were compared against established distribution scores of the EATQ-R shyness and fear scales (Ellis and Rothbart, 2001). The sample was stratified such that approximately 2/3 of participants ($n = 85$) scored > 0.75 SDs above the mean on the parent- or adolescent-rated fearfulness (3.12 for parent-report, 3.48 for adolescent-report) or shyness scales (2.99 for parent-report, 3.16 for adolescent-report). All other participants ($n = 44$) scored below this cut-off and were within the normative range of shy/fearful temperament. Because stratification based on temperament measures was used to increase variability in social threat as part of the aims of the larger study, we do not report on potential individual differences related to temperament.

Exclusion criteria included: meeting DSM-5 criteria for a current or lifetime diagnosis of any anxiety disorder (except for specific phobia), obsessive-compulsive disorder, post-traumatic stress disorder, major depressive disorder, or any psychotic or autism spectrum disorder, as determined by the Kiddie-Schedule for Affective Disorders and Schizophrenia (KSADS-PL; Kaufman et al., 2016); IQ lower than 70, as assessed using the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999); a lifetime presence of a neurological or serious medical condition; the presence of any MRI contraindications; presence of head injury or congenital neurological anomalies (based on parent report); acute suicidality; and taking medications that affect the central nervous system and hormonal function (e.g., selective serotonin reuptake inhibitors, steroid medication). Stimulants were permitted if use was discontinued for 36 h prior to the scan.

Data for the current analysis focused on the baseline assessment and included participants according to the following criteria: good quality resting state fMRI data (see below for criteria), salivary assays to index basal levels of testosterone, and self-report measures of pubertal status anxiety symptoms per the Screen for Child Anxiety Related Emotional Disorders (Birmaher et al., 1997). From the 129 participants, 90 completed resting state scans at baseline. Of these 90 participants, 89 had Pubertal Development Scale (PDS) score and 83 had hormone data. Resting state scans of 18 participants were excluded due to excess motion (see below) and one was excluded due to an incidental finding.

Thus, a total of 70 participants (42 high in shy/fearful temperament; age: $M = 12.30$, $SD = 0.79$) were included in the analysis linking resting-state fronto-amygdala functional connectivity, anxiety symptoms, and at least one measure of puberty. Out of those 70 participants, 64 had hormone data. See Table 1 for sample characteristics.

2.2. Procedure

The study was approved by the University of Pittsburgh Human Research Protection Office. The study occurred in three visits to the lab. During their first visit to the lab, parents provided informed consent and youth provided informed assent to acknowledge their voluntary agreement to participate in the study. Following informed consent, participants completed the screening procedures that included administration of two subtests of the WASI (Vocabulary and Matrix Reasoning) and the KSADS-PL with the participant and the parent about their child. Participants completed the neuroimaging protocol during Visit 3 (generally 2 weeks following Visit 2 and they completed the questionnaires online using Qualtrics during Visit 2 (generally 2 weeks following Visit 1) or at home within 2 weeks of Visit 1. Participants were compensated for their time in the study.

Table 1
Descriptive statistics for sample demographics, pubertal development, and questionnaire measures.

Characteristic	Statistics
Age, mean (SD)	12.30 (0.79)
Sex, n (%)	70 (100%)
Female	
SES, n (%)	1 (1.4%)
$0 - $10,000	1 (1.4%)
$10,001 - $20,000	6 (8.6%)
$30,001 - $40,000	6 (8.6%)
$40,001 - $50,000	4 (5.7%)
$50,001 - $60,000	5 (7.1%)
$60,001 - $70,000	7 (10.0%)
$70,001 - $80,000	4 (5.7%)
$80,001 - $90,000	7 (10.0%)
$90,001 - $100,000	27 (38.6%)
$100,000 +	2 (2.9%)
N/A	
Race, n (%)	1 (1.4%)
Asian	6 (8.6%)
Biracial	10 (14.3%)
Black, not Hispanic	2 (2.9%)
Other	51 (72.9%)
White, not Hispanic	
Ethnicity, n (%)	4 (5.7%)
Hispanic	58 (82.9%)
Non-Hispanic	8 (11.4%)
N/A	
Puberty, mean (sd)	3.25 (0.86)
Adrenarcheal and	3.26 (1.13)
Gonadarcheal (PDSS)	3.23 (0.76)
Adrenarcheal (PDSA)	
Gonadarcheal (PDSG)	
Testosterone (pg/ml), mean (SD)	3.82 (0.35)
SCARED-C, mean (SD)	15.92 (11.26)
BMI, mean (SD)	19.69 (3.82)
Mean FD, mean (SD) [range]	0.22 (0.10) [0.08–0.54]
% Timepoints Removed, mean (SD)	0.26 (0.19)

Note: SD = standard deviation; SES = Socio-demographic status (i.e., parent-reported family income); PDSS = Pubertal Development Scale total score; PDSA = Pubertal Development Scale adrenarcheal score; PDSG = Pubertal Development Scale gonardarcheal score; SCARED-C = Screen for Child Anxiety-Related Emotional Disorders – Child report; BMI = Body Mass Index. FD = Framewise Displacement

2.3. Measures

2.3.1. Risk status at recruitment

Risk status was assessed using the EATQ-R (Ellis and Rothbart, 2001). The EATQ-R consists of 12 scales. The current study examined only the shyness and fearfulness scales. Both parent-report and participant self-report scores on the EATQ-R shyness and fear scales were considered to determine risk-status (either "high-risk" or "low-risk"). For the full sample (n = 129), internal consistency for the EATQ-R shyness scale was moderate for adolescent self-report (Cronbach's α = 0.75) and high for parent report (α = 0.85). Internal consistency for the EATQ-R fear scale was low for adolescent self-report (α = 0.47) and parent report (α = 0.66).

To determine risk-status, participants were compared against established distribution scores of the EATQ-R (Ellis and Rothbart, 2001). Participants with a score more than 0.75 SDs above the established mean on the fear or shyness scales on either child or parent report were accepted into the study as part of the high-risk group, and participants with a score less than 0.75 SDs above the mean on the fear and shyness scales were accepted as part of the low-risk group.

2.3.2. Demographic variables

See Table 1 for descriptive information about the sample. Distribution of PDS scores and testosterone levels can be found in the Supplemental Materials (see Figs. S1 a-c and Fig. S2).

2.3.3. Pubertal development

Perceived pubertal status. Participants reported their perceived pubertal development using the Pubertal Development Scale (PDS) (Petersen et al., 1988). The PDS includes items that assess physiological changes in body hair, skin, and height. The female version includes additional items pertaining to breast development and menstruation whereas the male version includes items about facial hair growth and voice change. Each item has four response options ranging from 1 = not yet started to 4 = seems complete. The response option for the item about menstruation was 0 = no, 1 = yes. The PDS has good internal consistency (Cronbach's $\alpha = 0.91–0.96$) and test-retest reliability (ICC = 0.81–0.93). A scoring algorithm converts the PDS to the Tanner metric (1−5), and provides a sensitive way to differentiate between adrenarcheal and gonadarcheal physical changes, respectively (Shirtcliff et al., 2009). Three scores were computed: an overall PDS score (PDSS), an adrenarcheal development score (PDSA), and gonadarcheal development score (PDSG).

Testosterone. Circulating levels of testosterone were assessed using salivary assays with commercially available immunoassays from Salimetrics LLC published protocols. During their initial visit in the lab, participants and their parents were provided with instructions for saliva collection, storage, and shipping protocols as well as home saliva collection kits, which included a cardboard box that contained four pre-labeled vials, a saliva collection diary, an ice pack, and a plastic bag. Participants provided saliva samples at home immediately upon awakening (typically between 7 am and 9 am) on 4 separate days over a four-week period (one sample per week) following the initial visit. The duration of 4 weeks was requested to minimize the influence of menstrual cyclicality because pubertal hormones cycle even in premenarcheal girls (Biro et al., 2014). The four saliva samples were collected approximately within 6 weeks of the MRI scan. Participants expectorated approximately 1.8 ml of saliva via passive drool into a 2 ml cryovial using short straws. To prevent (blood) contamination of the saliva samples, participants were asked to avoid (1) brushing their teeth or eating a major meal for at least 1 h prior to collection, (2) eating anything acidic or high-sugar within 20 min before collection, and (3) consuming something that stimulated the production of saliva (e.g., chewing gum). They were also asked to rinse their mouth with water about 10 min prior to collection. Participants then completed a brief saliva diary. With supervision from parents, participants were instructed to store the samples in their home-freezer immediately upon collection and then to return the vials, on ice-packs to minimize freeze-thaw cycles, at the visit.

Saliva samples were stored in an ultra-cold − 80°C freezer. Samples were batch shipped overnight on dry ice to the Stress Physiology Investigative Team laboratory and remained frozen at − 80°C until the day of assay. Commercially available and well-validated salivary enzyme-immunoassays kits (www.salimetrics.com) were used for each respective hormone. Samples were assayed in duplicate and values that disagreed by more than Optical Density coefficient of variation (CV)= 10% were re-run. The averaged intra-assay CV was M= 1.20, SD= 0.23. All hormones for a participant were assayed on the same day to minimize freeze-thaw cycles, and all samples from a participant were assayed on the same plate to minimize variation in hormone concentrations across plates. The average inter-assay CVs across plates was 17.26. As expected, the distributions of hormones were positively skewed, with skewness of 7.16 (SE=1.57). Therefore, hormones over 2.5 SD of the sample mean (< 1.96%), were winsorized to 2.5 SD values, and then scores were log-transformed, as is common in prior literature (Dismukes et al., 2015). Few samples (n = 13) were below the level of detection of the assay and 3 were excluded for having low levels of testosterone for all four samples.

To derive a single basal score, we extracted Empirical Bayesian (EB) estimation scores using Hierarchical Linear Modeling (HLM™ v.7, Scientific Software International, Inc.). Prior research (Ladouceur et al., 2019; Shirtcliff and Ruttle, 2010) has demonstrated that use of EB

estimates increases reliability of salivary hormones. The EB estimates "shrink" the basal scores toward the individual (level 1) and population mean (level 2), thereby minimizing the influence of a particularly high or low sample without excluding the data. The intra-class correlation (ICC), which was computed to describe fluctuation of hormone levels over the four weeks, shows that 61% of the total variance is shared across the four samples, suggesting moderate stability in testosterone levels across weeks. Finally, 64 out of the 70 subjects had valid testosterone data.

2.3.4. Clinical assessments

The K-SADS-PL (parent and child interviews; Kaufman et al., 2016) was administered to each participant and her primary caregiver separately by trained interviewers (master's/doctoral level clinicians) to determine current and past DSM-5 diagnoses for each participant for eligibility purposes. The Screen for Child Anxiety-Related Emotional Disorders (SCARED; Birmaher et al., 1999) was used to assess child symptoms of anxiety. The SCARED included 41-items whereby participants respond to each item using a 3-point Likert scale (0: almost never, 1: sometimes, 2: often).

2.3.5. fMRI acquisition

Neuroimaging data were acquired using a 3 Tesla Siemens Prisma magnet with a 64-channel phase array coil. Anatomical images covering the entire brain were acquired first using a three-dimension magnetization-prepared rapid gradient-echo T1-weighted sequence (repetition time [TR]: 2300 ms, echo time [TE]: 3.93 ms, flip angle: 9∘, inversion time [TI]: 900 ms, field of view: 256 × 256 mm, 256 slices, voxel size: 1 mm^3). Participants completed two fMRI tasks designed to examine neural substrates of social threat processing, a reward-processing task, and two 6-minute resting state sessions during which they were instructed to look at a fixation cross in the middle of the screen. Task stimuli were projected onto a color, high-resolution LCD screen in front of the scanner bed and viewed in a mirror mounted on the head coil. Head movement was constrained by foam padding. Functional images were acquired using a multiband gradient-echo T2 * -weighted EPI sequence (multiband acceleration factor: 3, 60 slices, TR: 1500 ms, TE: 30 ms, flip angle: 55°, echo spacing: 0.60 ms, field of view: 221 × 221 ×138 mm, voxel dimensions: 2.3 mm × 2.3 mm×2.3 mm). Field maps were acquired using gradient echo planar imaging sequence for correction of field distortions in the functional images with the following parameters: TR: 590 ms, TE1: 4.92 ms, TE2: 7.38 ms, voxel size: 2.3 × 2.3 × 2.3 mm, flip angle: 60°.

2.4. fMRI processing

fMRI data were preprocessed using fMRIPrep (v1.5.3; Esteban et al., 2019), which includes EPI to T1w coregistration, susceptibility artifact correction, normalization to MNI space, and estimation of motion parameters. Details can be found in the Supplemental Materials. Following minimal preprocessing, several additional processing steps were applied. Following functional connectivity (FC) processing guidelines (Ciric et al., 2017), 36 parameter nuisance regression (6 degrees of motion, global signal, white matter and CSF, with temporal, quadratic, and quadratic temporal derivatives) along with high-pass spectral filtering at 0.008 mHz were applied. Bandpass filtering was also applied to the regressor matrix as recommended (Hallquist et al., 2013). Framewise displacement (FD; Power et al., 2012) was used as a measure of motion contamination of individual volumes. Volumes with FD greater than 0.3 were removed, as were volumes immediately adjacent to contaminated volumes. To combine scrubbing and spectral filtering, spectral interpolation based on the XCP pipeline (Ćirić et al., 2020) was used. All postprocessing steps were implemented using a customized processing pipeline (clpipe; Henry et al., 2020). Additionally, scans that exceed a mean FD of 0.6 or lost more than 40% of available volumes due to removal, were excluded from subsequent analyses.

2.4.1. Regions-of-interest (ROI) mask and extraction

The set of ROIs used to characterize both the vmPFC and the bilateral amygdala was constructed by combining the 6 ROI vmPFC parcellation from Chase et al. (2020) (Chase et al., 2020) with the right and left amygdala ROIs of the AAL3 atlas (Rolls et al., 2020). The 6 ROI vmPFC parcellation is a functional parcellation of the vmPFC based on a meta-analysis of both task and resting state studies contained in the BrainMap database (Fox and Lancaster, 2002).

Dividing the vmPFC into these subregions allows for more specificity in analyzing the vmPFC-amygdala connectivity characteristics than keeping the vmPFC as a single larger ROI. The amygdala ROIs are anatomically defined. Fig. 1 shows the ROI mask overlayed on the standard MNI anatomical image. Voxels within each ROI were averaged to form 8 total ROI timeseries for each participant. These timeseries were detrended and standardized to a mean of 0 and a standard deviation of 1.

2.4.2. Susceptibility artifacts and data loss

Due to dropout caused by the sinus cavities, 50% of our sample had 2 unusable vmPFC ROIs (ROIs 1 and 6); these were the most ventral, and therefore closest to the air cavities of the sinuses. Because of the amount of data loss, we did not examine the connectivity between these ROIs and the bilateral amygdala ROIs. We did include ROI 1 and ROI 6, when available, in the computation of the partial correlations for other ROIs.

2.5. Statistical analyses

2.5.1. Functional connectivity using partial correlations

The functional connectivity between two ROIs was operationalized as the lag-0 partial correlation. Partial correlations control for the confounding relations between other variables in the analysis, and functional connectivity measured with these correlations can be interpreted as the relation unique to the two ROIs in question. Partial correlations were Fisher Z transformed before subsequent analyses.

2.5.2. Moderated mediation

To examine how both testosterone and pubertal development (adrenarcheal and gonadarcheal processes) are related to anxiety symptoms via the vmPFC-amygdala resting state functional connectivity, as well as how testosterone and pubertal development could moderate the relation between vmPFC-amygdala connectivity and anxiety symptoms, we used moderated mediation models (Preacher et al., 2007) as depicted in Fig. 2.

The moderated mediation model proposed is like a standard three-variable mediation model, but also allows for testosterone or adrenarcheal development or gonadarcheal development to moderate the relation between vmPFC-amygdala connectivity and anxiety symptoms. This allows one to examine how different levels of testosterone or pubertal development impact the direct relation between vmPFC-amygdala connectivity and anxiety symptoms, as well as how the indirect relation from testosterone or pubertal development is self-moderated by testosterone or adrenarcheal development or gonadarcheal development, respectively.[6] All models controlled for age. Additionally, a series of sensitivity analyses were performed and are presented in the Supplemental Materials. These include analyses

[6] Regarding the use of mediation on this cross-sectional data, it is important to note that we do not have a true experimental design nor temporal precedence at the correct timescale to help infer the direction of the effects and as such, we must rely upon the theoretical position that puberty to brain to anxiety symptoms is a reasonable causal structure here. However, it is likely that the statistical association between our variables of interest are in part due to other causal mechanisms, such as anxiety impacting development or functional connectivity. Thus, it is recommended to avoid drawing causal conclusions from the current findings.

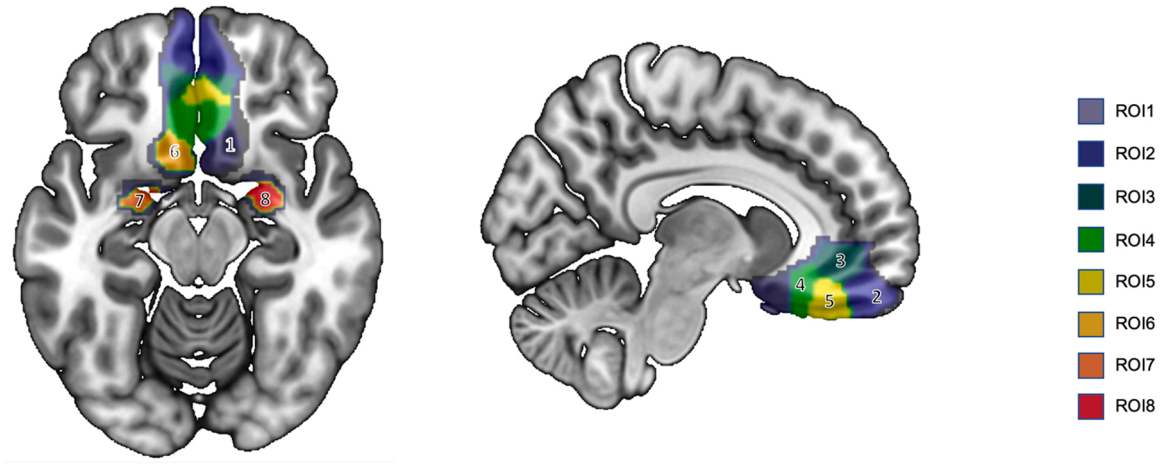

Fig. 1. Region-of-interest mask of the ventromedial prefrontal cortex (ROI 1–6) and the amygdala (ROI 7 and 8) overlayed on the standard Montreal Neurological Institute (MNI) anatomical image.

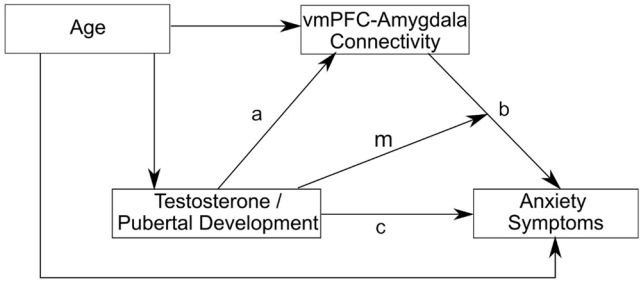

Fig. 2. Moderated Mediation Model Path Diagram. Boxes represent observed variables and arrow indicate a directed linear relation. Separate models were computed with testosterone, adrenarcheal processes assessed using the Pubertal Development Scale (PDS) adrenarche composite score, and gonadarcheal processes assessed using the PDS gonadarche composite score.

controlling for in-scanner motion, analyses examining the change in inference when ROI 1 and 6 were not considered for any participant when calculating partial correlations, and analyses using a moderation only model. These sensitivity analyses are discussed at the end of the results section below.

Thus, 24 models were fit (8 vmPFC-amygdala connectivity variables with testosterone or adrenarcheal or gonadarcheal processes). To allow for comparability between models, all variables were Z-scored before models were fit. As the moderating effect of testosterone or pubertal development (adrenarcheal or gonadarcheal) is of primary interest, we present and report only those models with a significant moderating effect. Other fit models are available in the Supplemental Materials. All models are fit in R (R CoreTeam, 2019) using the lavaan package (Rosseel, 2012). Importantly, due to the exploratory nature of these analyses and the moderately-sized sample, multiple comparison corrections were not applied. This limitation is discussed in the Discussion.

3. Results

3.1. Descriptive statistics

Descriptive statistics for the current sample are displayed in Table 1.

3.2. Moderation-mediation models

Two models contained a significant moderating effect for vmPFC ROI 3 – Right Amygdala: one for testosterone and one for adrenarcheal processes (see Fig. 3A). One model for gonadarcheal processes showed a significant moderating effect on vmPFC ROI 2 – Left Amygdala (see Fig. 4A). Figs. 3–4 show the path diagrams for these models along with effect estimates, simple slopes, and indicators of statistical significance. Full outputs for these models are available in the Supplemental Materials.

For models with testosterone levels and those with adrenarcheal development scores, high levels of testosterone or more advanced adrenarcheal development serve to potentiate the negative relation between vmPFC ROI 3 – Right Amygdala connectivity on anxiety, as evidenced by significant moderation effects (Testosterone: $\beta = -0.269$, $SE = 0.117, p < .05$; Adrenarcheal Development: $\beta = -0.256, SE = 0.116, p < .05$). Simple slope analysis revealed that stronger vmPFC ROI 3 – Right Amygdala connectivity was associated with fewer anxiety symptoms either at elevated levels of testosterone or more advanced adrenarcheal development (Simple slopes for Testosterone: $\beta = -0.373, SE = 0.170, p < .05$; Adrenarcheal Development: $\beta = -0.431, SE = 0.167, p < .05$). In addition, there was a marginal direct effect of testosterone on vmPFC ROI 3 – Right Amygdala connectivity ($\beta = -0.226, SE = 0.122, p = .063$).

As depicted in Fig. 4, we also found a significant moderated mediation model for gonadarcheal development where more advanced development moderated the relation between vmPFC R01 2 – Left Amygdala connectivity and anxiety symptoms ($\beta = -0.249, SE = 0.121, p < .05$). Simple slopes analysis revealed, as with the testosterone and adrenarcheal development models, that stronger vmPFC R0I 2 – Left Amygdala connectivity was associated with fewer anxiety symptoms at more advanced gonadarcheal development ($+1SD$Gonadarcheal Development : $\beta = -0.249, SE = 0.171, p < .05$).

3.3. Sensitivity analyses

All supplementary analyses are presented in the Supplemental Materials, and the overall findings (and how they compare to the reported findings) are briefly discussed here. An analysis of how in-scanner motion (as FD) relates to our primary variables of interest showed that FD was significantly correlated with ROI 3 - R Amygdala connectivity (implicated in testosterone and adrenarcheal processes). Refitting the models while controlling for mean FD showed the same pattern of significant findings with minimal change in either the effect estimates or standard errors, suggesting that in-scanner motion is not confounding our relations of interest.

Next, models, which made use of partial correlations calculated without ROI 1 or ROI 6 for the entire sample, were fit to test the sensitivity of our inference to the mixed nature of the sample. Not controlling for ROI 1 and ROI 6 in half the sample led to an attenuation

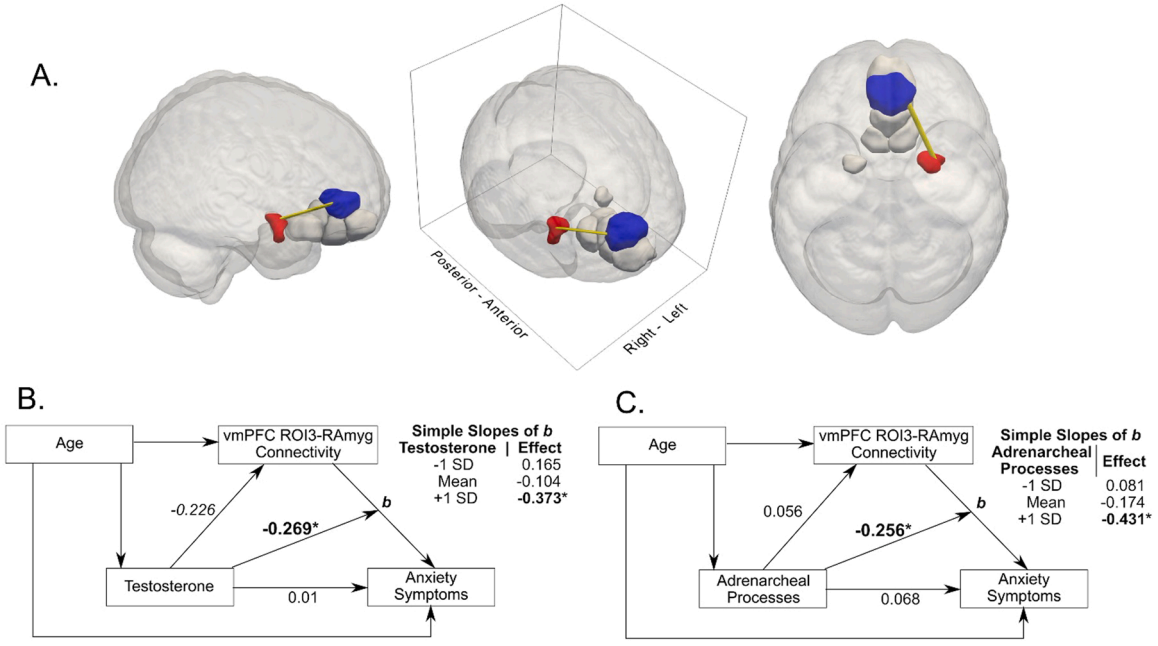

Fig. 3. A. Depiction of vmPFC ROI 3 – Right Amygdala functional connectivity. B. Moderation Mediation Model for Testosterone. **Bold*** indicates that the effect is significant ($p < .05$), with *italics* indicating marginal significance ($p < .10$). Simple slopes for the vmPFC ROI 3 – amygdala connectivity to anxiety symptoms effect (b) are presented for + 1 SD mean and − 1 SD testosterone. C. Moderation Mediation Model for Adrenarcheal Processes. **Bold*** indicates that the effect is significant ($p < .05$), with *italics* indicating marginal significance ($p < .1$). Simple slopes for the vmPFC ROI 3 – Right Amygdala connectivity to anxiety effect (b) are presented for + 1 SD, mean and − 1 SD adrenarcheal processes. Note: Due to standardization, all effect estimates are in β metric.

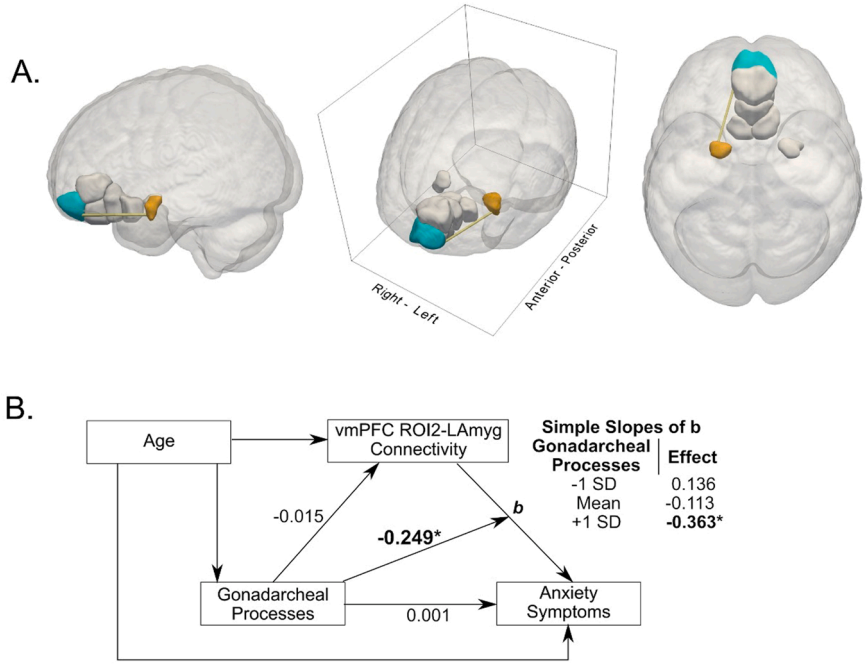

Fig. 4. vmPFC ROI 2 – Left Amygdala and Gonadarcheal development moderated mediation. A. Depiction of vmPFC ROI 2 – Left Amygdala functional connectivity. B. Moderation Mediation Model for Gonadarcheal Processes. **Bold*** indicates that the effect is significant ($p < .05$). Simple slopes for the connectivity-anxiety effect (b) are presented for + 1 SD, mean and − 1 SD gonadarcheal processes. Note: Due to standardization, all effect estimates are in β metric.

of all significant effect estimates such that the moderating effect of testosterone (on the relation between ROI 3 - R Amygdala connectivity and anxiety) and the moderating effect of gonadarcheal development (on the relation between ROI 2 – L Amyg connectivity to anxiety) remained significant. The significant moderating relation of adrenarcheal development (on the relation between ROI 3 - R Amygdala connectivity and anxiety) became marginally significant. This pattern of attenuation (rather than exaggeration) in the effect size estimates and increases in the effect size standard errors is due to not using available ROI 1 and 6 information when calculating partial correlations. Consequently, partial correlations became noisier and slightly biased, which in turn, resulted in attenuated effect estimates and inflated standard errors. We discuss the limitations of our approach and implications for future work in the Discussion section.

Additionally, moderation only models were fit. Only the moderating effect of testosterone on ROI 3 - R Amygdala connectivity's relation with anxiety was significant; however, the other two reported effects for adrenarcheal and gonadarcheal processes were marginally significant and of approximately the same magnitude as in the moderated-mediation models.

Finally, we evaluated the overall fit of moderated-mediation, moderation only, and mediation only models using Akaike information criterion (AIC) and Bayesian information criterion (BIC). This provides an additional way of assessing which model is the most optimal and parsimonious. We report the AIC and BIC in the Supplementary Materials. For our significant testosterone model (ROI 3 - R Amygdala), AIC selects the moderated-mediation model, while BIC selects the moderation only model, albeit by less than 2 points. This pattern of results is consistent with the marginally significant relation between testosterone and ROI 3 - R Amygdala connectivity. For our significant adrenarcheal development and gonadarcheal development models, both AIC and BIC selected the moderation only model, which corresponds to the lack of significant direct relations between the developmental measure and connectivity. Overall, these model comparisons support findings of testosterone and developmental processes moderating the relation between vmPFC-amygdala connectivity and anxiety, and suggest that more data are needed to assess for a potential mediating relation between testosterone, brain connectivity, and anxiety.

4. Discussion

In the current study, we used moderation-mediation models to examine the relationship between testosterone/pubertal development, vmPFC-amygdala resting state functional connectivity, and anxiety symptoms in a sample of early adolescent girls varying in temperamental risk for anxiety disorders. We found that vmPFC-amygdala connectivity was associated with anxiety symptoms only at higher levels of testosterone and more advanced pubertal development. Specifically, we found that testosterone and adrenarcheal processes moderate the *negative* association between the right amygdala and vmPFC ROI 3 connectivity and anxiety symptoms. In addition, gonadarcheal processes moderate the *negative* association between left amygdala and vmPFC ROI 2 connectivity and anxiety symptoms. In other words, within girls with themselves more advanced puberty or who had higher levels of testosterone, weaker fronto-amygdala connectivity was associated with more anxiety symptoms (or, conversely, within girls more pubertally advanced or with higher levels of testosterone, stronger fronto-amygdala connectivity was associated with less anxiety). Because testosterone is implicated in both adrenarcheal and gonadarcheal processes, especially in females as testosterone is mostly produced by adrenal glands (Byrne et al., 2017), it is not surprising that the pattern of findings was similar across indices of pubertal development.

Our findings support our hypothesis regarding the moderating role of testosterone levels on the negative association between vmPFC-amygdala functional connectivity and anxiety symptoms. They are consistent with Spielberg et al.'s (2015) report of increases in pubertal testosterone concentrations over an approximately 2-year period associated with reduced OFC-amygdala coupling to angry/fearful faces compared to neutral faces/shapes in a typically-developing mixed-sex sample of 11–15-year-olds (Spielberg et al., 2015). Interestingly, while findings were significant for both sexes in that study, the authors noted that the range of change in testosterone levels was smaller in female participants suggesting that girls may be more sensitive to the modulatory effects of testosterone on this circuitry. In exploratory analyses, Spielberg and colleagues also observed reduced OFC-amygdala coupling over time to be associated with more withdrawal temperament traits assessed using the approach/withdrawal temperament subscale of the Dimensions of Temperament Survey (DOTS; Windle and Lemer, 1986) – though no interactions with testosterone were found. Such modulating effects of testosterone on fronto-amygdala connectivity – reducing connectivity between these two regions – is supported by serum testosterone administration studies in adult women. For instance, using a double-blind placebo-controlled design researchers reported that administration of testosterone in adult women led to increased activation to threat-related stimuli in both the amygdala and OFC (Hermans et al., 2008; van Wingen et al., 2009) and reduced OFC-amygdala coupling in a sample of middle-aged women (age 37–50) processing emotional faces (Van Wingen et al., 2010). Another study using a double-blind placebo-controlled design in a sample of young women (ages 18–37) reported that administration of testosterone was linked to decreases in the magnitude of OFC-amygdala coupling to escapable (vs. inescapable) threat; no differences were observed during rest (Heany et al., 2018). Taken together, the above findings suggest that fronto-amygdala functional connectivity during emotion processing tasks or at rest is less optimal (more positive or reduced coupling) in the context of high levels of testosterone. We speculate that girls, especially those at temperamental risk of anxiety disorders, may be particularly neurobiologically more sensitive to the modulatory effects of testosterone on vmPFC-amygdala connectivity and that such sensitivity may be linked to the emergence or rise of anxiety symptoms given the regulatory role of vmPFC on amygdala activity. It remains to be determined, however, to what extent our findings would generalize to other samples of adolescent girls recruited from the community with higher or low risk of developing anxiety disorders.

Whether the moderating effects of pubertal development was stronger for some subregions of the vmPFC and their connectivity with the amygdala was unknown. We addressed this gap by using a vmPFC six-cluster parcellation (Chase et al., 2020) to explore puberty-related connectivity between the amygdala and subregions of the vmPFC. We found that testosterone and adrenarcheal processes were related to connectivity between right amygdala and vmPFC ROI 3 – an ROI that includes regions implicated in regulatory processes (Chase et al., 2020) but not the OFC as previously reported in task-related studies (Spielberg et al., 2015). According to Chase and colleagues, the vmPFC ROI 3 sits directly anterior to area 14 m. Over 80% of voxels in this cluster overlaps with the following bilateral Brodmann areas: 14 m, which broadly corresponds with large parts of the vmPFC, area 24 – ventral anterior cingulate cortex (ACC), and area 32 with the rostral/dorsal ACC. These regions are comprised of several layers of pyramidal cells varying across layers in neuronal granularity, (Mackey and Petrides, 2014) suggesting that each of these large structures can likely be further parcellated based on cellular morphology into multiple subregions. Functionally, they are thought to be implicated in decision-making, (Smith et al., 2010) regulating affective responses (Suzuki and Tanaka, 2021) likely by downregulating amygdala activity, (Motzkin et al., 2015) and signaling social cognitive information regarding the states of others (Apps et al., 2016). In contrast to the vmPFC ROI 3 cluster, approximately 36% of voxels in vmPFC ROI 2 overlap with regions corresponding to the JuBrain atlas labels of areas 14 m and 11 and primarily associated with olfactory and gustation (Chase et al., 2020). Interestingly, an in-situ hybridization study in rats investigating the distribution of androgen and estrogen receptor mRNA-containing cells reported that the greatest density of these cells—which were almost exclusively neurons—were localized to the hypothalamus, amygdala nuclei, and the BNST but also included cortical olfactory regions and in the main and accessory olfactory bulbs (Simerly et al., 1990). Together, these findings suggest that amygdala connectivity with these subregions of the vmPFC is associated with levels of anxiety, particularly in girls who are more advanced in pubertal development. Future research investigating the mechanisms underlying puberty-specific development of these specific vmPFC-amygdala pathways and how alterations in such development contribute to trajectories of anxiety symptoms in girls is warranted to deepen our understanding of pathophysiology of anxiety disorders.

One mechanism could be via changes in the sensitivity of sex steroid hormone receptors in these vmPFC subregions or their connections with the amygdala. The amygdala has a high density of androgen receptors

(Simerly et al., 1990)—sensitive to changing levels of testosterone during puberty—and possibly increasing amygdala reactivity in the context of protracted maturation of ventromedial prefrontal cortical inhibitory pathways (Gee et al., 2013; Selleck et al., 2018; Wu et al., 2016). Given evidence that testosterone impacts dopaminergic function (Marowsky et al., 2005), another mechanism could be via the effects of testosterone on vmPFC signals in the amygdala while prioritizing other inputs to the amygdala (Grace and Rosenkranz, 2002), which could contribute to an anxiety response. Indirect effects are also possible such as perceived physical changes during puberty and their impact on girls' self-esteem, behavior, and reactivity to others' perception of them, which tax the emotion regulation circuitry thereby creating a vulnerability to the effects of pubertal hormones on this circuitry during a critical period of neuroplasticity (Larsen and Luna, 2018).

Because the girls in our sample varied in risk for anxiety disorders, it is possible that they may have been especially sensitive to the effects of testosterone in reducing fronto-amygdala connectivity in ways that may increase affect dysregulation and risk of anxiety. This interpretation is inconsistent, however, with findings from a study with a mixed-sex sample of typically developing children (mean age 9.53 years) showing that in boys, but not girls, testosterone levels were positively associated with anxiety symptoms and that this association was mediated by amygdala connectivity to visual cortical areas as well as the ACC (Barendse et al., 2018). Such discrepancy in findings could be attributed to differences in the age of the samples, with the Barendse et al. (2018) participants being younger and thus possibly not engaging fronto-amygdala circuitry during emotion processing as much as older participants in other studies. Indeed, longitudinal analyses with the same sample revealed that more advanced adrenarcheal development at baseline was associated with increases in anxiety symptoms over time and that this association was mediated by more positive (weaker) amygdala to lateral prefrontal cortex connectivity (across time) during fearful face processing (Barendse et al., 2020).

While we observed a marginally significant direct effect of testosterone on vmPFC ROI 3 – R Amygdala, the mediating role of reduced vmPFC-amygdala connectivity on the relationship between pubertal development and anxiety symptoms was not supported. These findings suggest that pubertal development, including increases in levels of testosterone, did not lead to reductions in vmPFC-amygdala connections and increases in levels of anxiety symptoms. A longitudinal design where within-subject changes in pubertal development over time would be associated with higher anxiety symptoms and that this relationship could be explained by a reduction in vmPFC-amygdala connectivity would be a more powerful test of this relationship.

4.1. Strengths and limitations

We present findings pertaining to the role of pubertal development, including testosterone concentrations and self-reported physical characteristics, in anxiety with a focus on the functioning of fronto-amygdala circuitry in early adolescent girls. The strengths of this study include examining both basal levels of testosterone and self-reported pubertal development considering separate processes of adrenarche and gonadarche in a well-characterized sample of early adolescent girls varying in level of temperamental risk of anxiety disorder. In addition, testosterone concentrations were derived using hierarchical linear modeling with four morning saliva assays collected over one month. Such an approach enabled us to extract the portion of variance in testosterone into a single Empirical Bayes estimate of basal hormone level accounting for non-linear fluctuations across the day and the month. Our use of moderated-mediation models with ROIs based on the functional parcellation of the vmPFC (Chase et al., 2020) provides an in-depth analysis of the connectivity of the amygdala with functional subregions of the vmPFC improving interpretation of findings through the mapping of these subregions to corresponding regions in rodent and non-human primate studies.

Some limitations are important to consider for future research. First, although early adolescent girls are an important population in which to study the role of pubertal maturation in the development of anxiety (Beesdo-Baum and Knappe, 2012), it remains unclear whether findings might extend to adolescent boys or whether they extend to non-cisgender youth (transgender, non-binary). Future research is needed to examine whether similar processes are present in at-risk boys and larger and more diverse samples. Second, the moderate sample size within this age range may have limited our ability to detect variability in pubertal development as a function of age and examine the specific effects of temperamental risk status. Third, the use of a cross-sectional design precludes drawing conclusions about the direction of the relationship between pubertal development, fronto-amygdala functional connectivity, and anxiety symptoms, as well as testing hypotheses related to the *rate* of pubertal maturation across visits. Fourth, the lack of directionality of the functional connectivity findings precludes our ability to determine whether more advanced pubertal maturation is linked reduced inhibitory vmPFC connections to the amygdala or reduced amygdala input into the vmPFC. Fifth, the exploratory nature of the analyses with respect to the specific subregions of the vmPFC, combined with the moderate sample size, raises the possibility that these findings may include false positive effects. While the findings are consistent with previous research and suggest differential involvement of different vmPFC subregions, future studies need to confirm the role of the specific subregions of the vmPFC in a larger sample. Sixth, results from the sensitivity analysis assessing how exclusion of data from ROI 1 and 6 impacted inference showed that upon removing ROI 1 and 6 from consideration entirely, the moderating effect of adrenarcheal development becomes marginally significant. This indicates that the reported moderating effect of adrenarcheal development, particularly ROI 3 - R Amygdala connectivity, should be replicated in future work, as the change in significance suggests that ROI 1 and/or 6 could potentially explain part of the observed relation. Additionally, future studies need to consider other factors that may have direct or indirect effects such as family environment (Thijssen et al., 2022) or socio-economic status (Barch et al., 2020). Future research should include multiple measures of pubertal maturation and fronto-amygdala function (resting state and task) over several time points to determine to what extent exposure to testosterone impacts fronto-amygdala functioning. There is also a need to identify the molecular mechanisms by which levels of pubertal hormones such as testosterone influence fronto-amygdala connectivity in ways that contribute to trajectories of anxiety symptoms in youth. Finally, we note that our study only considered sex assigned at birth but that future research should incorporate data on gender identity, given that transgender and non-binary adolescents are likely to be at an even greater risk of developing anxiety symptoms during adolescence (de Vries et al., 2011).

5. Conclusions

Findings from the current study show that vmPFC-amygdala resting state functional connectivity is associated with anxiety symptoms only in early adolescent girls more advanced in adrenarcheal and/or gonadarcheal development and with higher levels of testosterone. Specifically, only in girls perceiving to be more advanced in puberty and having higher levels of testosterone is weaker fronto-amygdala connectivity associated with more anxiety symptoms (or, conversely, only in girls perceiving to be more advanced in puberty and having higher levels of testosterone is stronger fronto-amygdala connectivity associated with less anxiety). The moderating role of perceived pubertal maturation and testosterone varies as a function of vmPFC subregions suggesting potentially different neurodevelopmental mechanisms in rise of anxiety in at-risk girls. Current findings add to the sparse literature on the role of pubertal maturation and pubertal hormones on the functioning of neural circuitry implicated in the development of anxiety symptoms in girls during early adolescence. Longitudinal studies

examining puberty-related neurodevelopmental mechanisms underlying trajectories of anxiety symptoms in male and female youth are also needed to deepen our understanding of the sex differences in the development of internalizing disorders that emerge during adolescence.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request. Behavioral and neural data from this study are not currently publicly available but are available for a subset of study participants who consented to the public use of their data upon request. Please contact the corresponding author for more details.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dcn.2023.101236.

References

Abend, R., Swetlitz, C., White, L.K., Shechner, T., Bar-Haim, Y., Filippi, C., Kircanski, K., Haller, S.P., Benson, B.E., Chen, G., Leibenluft, E., Fox, N.A., Pine, D.S., 2020. Levels of early-childhood behavioral inhibition predict distinct neurodevelopmental pathways to pediatric anxiety. Psychol. Med 50 (1), 96–106. https://doi.org/10.1017/s0033291718003999.

Apps, M.A., Rushworth, M.F., Chang, S.W., 2016. The anterior cingulate gyrus and social cognition: tracking the motivation of others. Neuron 90 (4), 692–707. https://doi.org/10.1016/j.neuron.2016.04.018.

Banks, S.J., Eddy, K.T., Angstadt, M., Nathan, P.J., Phan, K.L., 2007. Amygdala–frontal connectivity during emotion regulation. Soc. Cogn. Affect. Neurosci. 2 (4), 303–312. https://doi.org/10.1093/scan/nsm029.

Barch, D.M., Shirtcliff, E.A., Elsayed, N.M., Whalen, D., Gilbert, K., Vogel, A.C., Tillman, R., Luby, J.L., 2020. Testosterone and hippocampal trajectories mediate relationship of poverty to emotion dysregulation and depression. Proc. Natl. Acad. Sci. USA 117 (36), 22015–22023. https://doi.org/10.1073/pnas.2004363117.

Barendse, M.E.A., Simmons, J.G., Byrne, M.L., Patton, G., Mundy, L., Olsson, C.A., Seal, M.L., Allen, N.B., Whittle, S., 2018. Associations between adrenarcheal hormones, amygdala functional connectivity and anxiety symptoms in children. Psychoneuroendocrinology 97, 156–163. https://doi.org/10.1016/j.psyneuen.2018.07.020.

Barendse, M.E.A., Simmons, J.G., Patton, G., Mundy, L., Byrne, M.L., Seal, M.L., Allen, N. B., Whittle, S., 2020. Adrenarcheal timing longitudinally predicts anxiety symptoms via amygdala connectivity during emotion processing, 739-748.e732 J. Am. Acad. Child Adolesc. Psychiatry 59 (6). https://doi.org/10.1016/j.jaac.2019.04.018.

Barendse, M.E.A., Byrne, M.L., Flournoy, J.C., McNeilly, E.A., Guazzelli Williamson, V., Barrett, A.Y., Chavez, S.J., Shirtcliff, E.A., Allen, N.B., Pfeifer, J.H., 2022. Multimethod assessment of pubertal timing and associations with internalizing psychopathology in early adolescent girls. J. Psychopathol. Clin. Sci. 131 (1), 14–25. https://doi.org/10.1037/abn0000721.

Barzilay, R., White, L.K., Moore, T.M., Calkins, M.E., Taylor, J.H., Patrick, A., Huque, Z. M., Young, J.F., Ruparel, K., Pine, D.S., Gur, R.C., Gur, R.E. Association of anxiety phenotypes with risk of depression and suicidal ideation in community youth. *Depression and Anxiety*, *n/a*(n/a). https://doi.org/10.1002/da.23060.

Beesdo-Baum, K., Knappe, S., 2012. Developmental epidemiology of anxiety disorders. Child Adolesc. Psychiatr. Clin. North Am. 21 (3), 457–478. https://doi.org/10.1016/j.chc.2012.05.001.

Birmaher, B., Khetarpal, S., Brent, D., Cully, M., Balach, L., Kaufman, J., Neer, S.M., 1997. The screen for child anxiety related emotional disorders (SCARED): Scale construction and psychometric characteristics. J. Am. Acad. Child Adolesc. Psychiatry 36, 545–553.

Birmaher, B., Brent, D., Chiapetta, L., Bridge, J., Monga, S., Baugher, M., 1999. Psychometric properties of the screen for child anxiety related emotional disorders scale (SCARED): A replication study. J. Am. Acad. Child Adolesc. Psychiatry 38, 1230–1236.

Biro, F.M., Pinney, S.M., Huang, B., Baker, E.R., Walt Chandler, D., Dorn, L.D., 2014. Hormone changes in peripubertal girls. J. Clin. Endocrinol. Metab. 99 (10), 3829–3835. https://doi.org/10.1210/jc.2013-4528.

Bittner, A., Goodwin, R.D., Wittchen, H.U., Beesdo, K., Höfler, M., Lieb, R., 2004. What characteristics of primary anxiety disorders predict subsequent major depressive disorder? , quiz 730 J. Clin. Psychiatry 65 (5), 618–626. https://doi.org/10.4088/jcp.v65n0505.

Blackford, J.U., Pine, D.S., 2012. Neural substrates of childhood anxiety disorders: a review of neuroimaging findings. Child Adolesc. Psychiatr. Clin. North Am. 21 (3), 501–525. https://doi.org/10.1016/j.chc.2012.05.002.

Bloom, P.A., VanTieghem, M., Gabard-Durnam, L., Gee, D.G., Flannery, J., Caldera, C., Goff, B., Telzer, E.H., Humphreys, K.L., Fareri, D.S., Shapiro, M., Algharazi, S., Bolger, N., Aly, M., Tottenham, N., 2022. Age-related change in task-evoked amygdala-prefrontal circuitry: a multiverse approach with an accelerated longitudinal cohort aged 4-22 years. Hum. Brain Mapp. 43 (10), 3221–3244. https://doi.org/10.1002/hbm.25847.

Brady, E.U., Kendall, P.C., 1992. Comorbidity of anxiety and depression in children and adolescents. Psychol. Bull. 111 (2), 244–255. ⟨http://www.apa.org⟩.

Britton, J.C., Lissek, S., Grillon, C., Norcross, M.A., Pine, D.S., 2010. Development of anxiety: the role of threat appraisal and fear learning. Depress Anxiety 28, 5–17.

Byrne, M.L., Whittle, S., Vijayakumar, N., Dennison, M., Simmons, J.G., Allen, N.B., 2017. A systematic review of adrenarche as a sensitive period in neurobiological development and mental health. Dev. Cogn. Neurosci. 25, 12–28. https://doi.org/10.1016/j.dcn.2016.12.004.

Casey, B.J., Heller, A.S., Gee, D.G., Cohen, A.O., 2019. Development of the emotional brain. Neurosci. Lett. 693, 29–34. https://doi.org/10.1016/j.neulet.2017.11.055.

Chase, H.W., Grace, A.A., Fox, P.T., Phillips, M.L., Eickhoff, S.B., 2020. Functional differentiation in the human ventromedial frontal lobe: a data-driven parcellation. Hum. Brain Mapp. 41 (12), 3266–3283. https://doi.org/10.1002/hbm.25014.

Chronis-Tuscano, A., Degnan, K.A., Pine, D.S., Perez-Edgar, K., Henderson, H.A., Diaz, Y., Raggi, V.L., Fox, N.A., 2009. Stable early maternal report of behavioral inhibition predicts lifetime social anxiety disorder in adolescence. J. Am. Acad. Child Adolesc. Psychiatry 48 (9), 928–935. https://doi.org/10.1097/CHI.0b013e3181ae09df.

Ciric, R., Wolf, D.H., Power, J.D., Roalf, D.R., Baum, G.L., Ruparel, K., Shinohara, R.T., Elliott, M.A., Eickhoff, S.B., Davatzikos, C., Gur, R.C., Gur, R.E., Bassett, D.S., Satterthwaite, T.D., 2017. Benchmarking of participant-level confound regression strategies for the control of motion artifact in studies of functional connectivity. Neuroimage 154, 174–187. https://doi.org/10.1016/j.neuroimage.2017.03.020.

Ćirić, R., Adebimpe, A., Cieslak, M., Rosen, A., sattertt, Tooley, U., Kahn, A., Tapera, T.M. , 2020. PennBBL/xcpEngine: compatible with fmriprep outputs of version 20.07 Version 1.2.1) Zenodo. https://doi.org/https://doi.org/10.5281/zenodo.3840960.

Cole, D.A., Peeke, L.G., Martin, J.M., Truglio, R., Seroczynski, A.D., 1998. A longitudinal look at the relation between depression and anxiety in children and adolescents. J. Consult. Clin. Psychol. 66 (3), 451–460. https://doi.org/10.1037/0022-006x.66.3.451.

Costello, E.J., Mustillo, S., Erkanli, A., Keeler, G., Angold, A., 2003. Prevalence and development of psychiatric disorders in childhood and adolescence. Arch. Gen. Psychiatry 60 (8), 837–844. ⟨http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12912767⟩.

Delevich, K., Klinger, M., Okada, N.J., Wilbrecht, L., 2021. Coming of age in the frontal cortex: the role of puberty in cortical maturation. Semin Cell Dev. Biol. 118, 64–72. https://doi.org/10.1016/j.semcdb.2021.04.021.

Dismukes, A.R., Shirtcliff, E.A., Hanson, J.L., Pollak, S.D., 2015. Context influences the interplay of endocrine axes across the day. Dev. Psychobiol. 57 (6), 731–741. https://doi.org/10.1002/dev.21331.

Dorn, L.D., Dahl, R.E., Woodward, H.R., Biro, F., 2006. Defining boundaries of early adolescence: a user's guide to assessing pubertal status and pubertal timing in research with adolescents. Appl. Dev. Sci. 10, 30–56.

Ellis, L.K., Rothbart, M.K. , 2001. Revision of the Early Adolescent Temperament Questionnaire. Biennial Meeting of the Society for Research in Child Development, Minneapolis, Minnesota.

Esteban, O., Markiewicz, C.J., Blair, R.W., Moodie, C.A., Isik, A.I., Erramuzpe, A., Kent, J. D., Goncalves, M., DuPre, E., Snyder, M., Oya, H., Ghosh, S.S., Wright, J., Durnez, J., Poldrack, R.A., Gorgolewski, K.J., 2019. fMRIPrep: a robust preprocessing pipeline for functional MRI. Nat. Methods 16 (1), 111–116. https://doi.org/10.1038/s41592-018-0235-4.

Etkin, A., Egner, T., Kalisch, R., 2011. Emotional processing in anterior cingulate and medial prefrontal cortex. Trends Cogn. Sci. 15, 85–93.

Fox, P.T., Lancaster, J.L., 2002. Opinion: mapping context and content: the BrainMap model. Nat. Rev. Neurosci. 3 (4), 319–321. https://doi.org/10.1038/nrn789.

Gabard-Durnam, L.J., Flannery, J., Goff, B., Gee, D.G., Humphreys, K.L., Telzer, E., Hare, T., Tottenham, N., 2014. The development of human amygdala functional connectivity at rest from 4 to 23 years: a cross-sectional study. Neuroimage 95, 193–207. https://doi.org/10.1016/j.neuroimage.2014.03.038.

Gee, D., Humphreys, K., Flannery, J., Goff, B., Telzer, E., Shapiro, M., Hare, T., Bookheimer, S., Tottenham, N., 2013. A developmental shift from positive to negative connectivity in human amygdala-prefrontal circuitry. J. Neurosci. 33, 4584–4593.

Ghashghaei, H.T., Hilgetag, C.C., Barbas, H., 2007. Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. Neuroimage 34 (3), 905–923. https://doi.org/10.1016/j.neuroimage.2006.09.046.

Goddings, A.-L., Mills, K.L., Clasen, L.S., Giedd, J.N., Viner, R.M., Blakemore, S.-J., 2014. The influence of puberty on subcortical brain development. Neuroimage 88, 242–251. https://doi.org/10.1016/j.neuroimage.2013.09.073.

Gold, A.L., Abend, R., Britton, J.C., Behrens, B., Farber, M., Ronkin, E., Chen, G., Leibenluft, E., Pine, D.S., 2020. Age differences in the neural correlates of anxiety disorders: an fMRI study of response to learned threat. Am. J. Psychiatry 177 (5), 454–463. https://doi.org/10.1176/appi.ajp.2019.19060650.

Goodwin, R.D., Fergusson, D.M., Horwood, L.J., 2004. Early anxious/withdrawn behaviours predict later internalising disorders. J. Child Psychol. Psychiatry 45 (4), 874–883. https://doi.org/10.1111/j.1469-7610.2004.00279.x.

Gotgay, N., Ordonez, A., Herman, D.H., Hayashi, K.M., Greenstein, D., Vaituzis, C., Lenane, M., Clasen, L., Sharp, W., Giedd, J.N., Jung, D., Nugent III, T.F., Toga, A.W., Leibenluft, E., Thompson, P.M., Rapoport, J.L., 2007. Dynamic mapping of cortical development before and after the onset of pediatric bipolar illness. J. Child Psychol. Psychiatry 48 (9), 852–862.

Grace, A.A., Rosenkranz, A., 2002. Regulation of conditioned responses of basolateral amygdala neurons. Physiol. Behav. 77, 489–493.

Guyer, A.E., Silk, J.S., Nelson, E.E., 2016. The neurobiology of the emotional adolescent: from the inside out. Neurosci. Biobehav. Rev. 70, 74–85. https://doi.org/10.1016/j.neubiorev.2016.07.037.

Hale, W.W., Raaijmakers, Q.A.W., Muris, P., Van Hoof, A., Meeus, W.H.J., 2009. One factor or two parallel processes? comorbidity and development of adolescent anxiety and depressive disorder symptoms. J. Child Psychol. Psychiatry 50 (10), 1218–1226. https://doi.org/10.1111/j.1469-7610.2009.02115.x.

Hallquist, M.N., Hwang, K., Luna, B., 2013. The nuisance of nuisance regression: spectral misspecification in a common approach to resting-state fmri preprocessing reintroduces noise and obscures functional connectivity. Neuroimage 0, 208–225. https://doi.org/10.1016/j.neuroimage.2013.05.116.

Hare, T.A., Tottenham, N., Galvan, A., Voss, H.U., Glover, G.H., Casey, B.J., 2008. Biological substrates of emotional reactivity and regulation in adolescence during an emotional Go-Nogo task. Biol. Psychiatry 63, 927–934.

Hariri, A., R, Mattay, V.S., Tessitore, A., Fera, F., Weinberger, D.R., 2003. Neocortical modulation of the amygdala response to fearful stimuli. Biol. Psychiatry 53, 494–501.

Heany, S.J., Bethlehem, R.A.I., van Honk, J., Bos, P.A., Stein, D.J., Terburg, D., 2018. Effects of testosterone administration on threat and escape anticipation in the orbitofrontal cortex. Psychoneuroendocrinology 96, 42–51. https://doi.org/10.1016/j.psyneuen.2018.05.038.

Henry, T., Duffy, K., Melwani, D., Cohen, J. , 2020. cohenlabUNC/clpipe: clpipe v1.3: GLM Update In (Version v1.3) Zenodo.

Hermans, E.J., Ramsey, N.F., Van Honk, J., 2008. Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. Biol. Psychiatry 63, 263–270.

Herting, M., Sowell, E., 2017. Puberty and structural brain development in humans. Front. Neuroendocrinol. 44, 122–137. https://doi.org/10.1016/j.yfrne.2016.12.003.

Jalbrzikowski, M., Larsen, B., Hallquist, M.N., Foran, W., Calabro, F., Luna, B., 2017. Development of white matter microstructure and intrinsic functional connectivity between the amygdala and ventromedial prefrontal cortex: associations with anxiety and depression. Biol. Psychiatry 82 (7), 511–521. https://doi.org/10.1016/j.biopsych.2017.01.008.

Jenkins, L.M., Andrewes, D.G., Nicholas, C.L., Drummond, K.J., Moffat, B.A., Phal, P.M., Desmond, P., 2018. Emotional reactivity following surgery to the prefrontal cortex. J. Neuropsychol. 12 (1), 120–141. https://doi.org/10.1111/jnp.12110.

Juraska, J.M., Sisk, C.L., DonCarlos, L.L., 2013. Sexual differentiation of the adolescent rodent brain: hormonal influences and developmental mechanisms. Horm. Behav. 64 (2), 203–210. https://doi.org/10.1016/j.yhbeh.2013.05.010.

Kaufman, J., Birmaher, B., Axelson, D., Perepletchikova, F., Brent, D., Ryan, N. , 2016. Schedule for Affective and Disorders and Schizophrenia for School Aged Children (6–18 Years): Kiddie-SADS-lifetime Version (K-SADS-PL DSM 5).

Ladouceur, C.D., 2012. Neural systems supporting cognitive-affective interactions in adolescence: the role of puberty and implications for affective disorders. Front. Integr. Neurosci. 6 https://doi.org/10.3389/fnint.2012.00065.

Ladouceur, C.D., Peper, J.S., Crone, E.A., Dahl, R.E., 2012. White matter development in adolescence: the influence of puberty and implications for affective disorders. Dev. Cogn. Neurosci. 2, 36–54.

Ladouceur, C.D., Kerestes, R., Schlund, M.W., Shirtcliff, E.A., Lee, Y., Dahl, R.E., 2019. Neural systems underlying reward cue processing in early adolescence: The role of puberty and pubertal hormones. Psychoneuroendocrinology 102, 281–291. https://doi.org/10.1016/j.psyneuen.2018.12.016.

Larsen, B., Luna, B., 2018. Adolescence as a neurobiological critical period for the development of higher-order cognition. Neurosci. Biobehav. Rev. 94, 179–195. https://doi.org/10.1016/j.neubiorev.2018.09.005.

Little, J.P., Carter, A.G., 2013. Synaptic mechanisms underlying strong reciprocal connectivity between the medial prefrontal cortex and basolateral amygdala. J. Neurosci. 33 (39), 15333–15342. https://doi.org/10.1523/jneurosci.2385-13.2013.

Mackey, S., Petrides, M., 2014. Architecture and morphology of the human ventromedial prefrontal cortex. Eur. J. Neurosci. 40 (5), 2777–2796. https://doi.org/10.1111/ejn.12654.

Madonna, D., Delvecchio, G., Soares, J.C., Brambilla, P., 2019. Structural and functional neuroimaging studies in generalized anxiety disorder: a systematic review. Braz. J. Psychiatry 41 (4), 336–362. https://doi.org/10.1590/1516-4446-2018-0108.

Marowsky, A., Yanagawa, Y., Obata, K., Vogt, K.E., 2005. A specialized subclass of interneurons mediates dopaminergic facilitation of amygdala function. Neuron 48 (6), 1025–1037. https://doi.org/10.1016/j.neuron.2005.10.029.

Monk, C.S., Nelson, E.E., McClure, E.B., Mogg, K., Bradley, B.P., Leibenluft, E., Blair, J.R., Chen, G., Charney, D.S., Ernst, M., Pine, D.S., 2008. Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. Arch. Gen. Psychiatry 65, 568–576.

Motzkin, J.C., Philippi, C.L., Wolf, R.C., Baskaya, M.K., Koenigs, M., 2015. Ventromedial prefrontal cortex is critical for the regulation of amygdala activity in humans. Biol. Psychiatry 77 (3), 276–284. https://doi.org/10.1016/j.biopsych.2014.02.014.

Murray, C.R., Simmons, J.G., Allen, N.B., Byrne, M.L., Mundy, L.K., Seal, M.L., Patton, G.C., Olsson, C.A., 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.

Myers-Schulz, B., Koenigs, M., 2012. Functional anatomy of ventromedial prefrontal cortex: implications for mood and anxiety disorders. Mol. Psychiatry 17 (2), 132–141. https://doi.org/10.1038/mp.2011.88.

O'Reilly, J.X., Woolrich, M.W., Behrens, T.E.J., Smith, S.M., Johansen-Berg, H., 2012. Tools of the trade: psychophysiological interactions and functional connectivity. Soc. Cogn. Affect. Neurosci. 7 (5), 604–609. https://doi.org/10.1093/scan/nss055.

Ochsner, K.N., Gross, J.J., 2005. The cognitive control of emotion. Trends Cogn. Sci. 9, 242–249.

Perlman, S.B., Pelphrey, K.A., 2010. Regulatory brain development: balancing emotion and cognition. Soc. Neurosci. 5 (5–6), 533–542. https://doi.org/10.1080/17470911003683219.

Petersen, A., Crockett, L., Richards, M., Boxer, A., 1988. A self-report measure of pubertal status: reliability, validity, and inital norms. J. Youth Adolesc. 17, 117–133.

Petrides, M., 2002. The mid-ventrolateral prefrontal cortex and active mnemonic retrieval. Neurobiol. Learn Mem. 78 (3), 528–538. https://doi.org/10.1006/nlme.2002.4107.

Pfaff, D., Keiner, M., 1973. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. J. Comp. Neurol. 151 (2), 121–158. https://doi.org/10.1002/cne.901510204.

Pfeifer, J.H., Allen, N.B., 2021. Puberty initiates cascading relationships between neurodevelopmental, social, and internalizing processes across adolescence. Biol. Psychiatry 89 (2), 99–108. https://doi.org/10.1016/j.biopsych.2020.09.002.

Phelps, E.A., LeDoux, J.E., 2005. Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 48 (2), 175–187. https://doi.org/10.1016/j.neuron.2005.09.025.

Phelps, E.A., Delgado, M.R., Nearing, K.I., LeDoux, J.E., 2004. Extinction learning in humans:role of the amygdala and vmPFC. Neuron 43 (6), 897–905.

Phillips, M.L., Ladouceur, C.D., Drevets, W.C., 2008. A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. Mol. Psychiatry 13 (9), 833–857.

Piekarski, D.J., Boivin, J.R., Wilbrecht, L., 2017. Ovarian hormones organize the maturation of inhibitory neurotransmission in the frontal cortex at puberty onset in female mice, 1735-1745.e1733 Curr. Biol. 27 (12). https://doi.org/10.1016/j.cub.2017.05.027.

Pine, D.S., Cohen, P., Gurley, D., Brook, J., Ma, Y., 1998. The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. Arch. Gen. Psychiatry 55, 56–64.

Power, J.D., Barnes, K.A., Snyder, A.Z., Schlaggar, B.L., Petersen, S.E., 2012. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 59 (3), 2142–2154. https://doi.org/10.1016/j.neuroimage.2011.10.018.

Preacher, K.J., Rucker, D.D., Hayes, A.F., 2007. Addressing moderated mediation hypotheses: theory, methods, and prescriptions. Multivar. Behav. Res 42 (1), 185–227. https://doi.org/10.1080/00273170701341316.

Price, R.B., Allen, K.B., Silk, J.S., Ladouceur, C.D., Ryan, N.D., Dahl, R.E., Forbes, E.E., Siegle, G.J., 2016. Vigilance in the laboratory predicts avoidance in the real world: a dimensional analysis of neural, behavioral, and ecological momentary data in anxious youth. Dev. Cogn. Neurosci. 19, 128–136. https://doi.org/10.1016/j.dcn.2016.03.001.

Quirk, G.J., Beer, J.S., 2006. Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. Curr. Opin. Neurobiol. 16, 723–727.

Quirk, G.J., Likhtik, E., Pelletier, J.G., Pare, D., 2003. Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. J. Neurosci. 23 (25), 8800–8807. ⟨http://www.jneurosci.org/cgi/content/abstract/23/25/8800⟩.

Reardon, L.E., Leen-Feldner, E.W., Hayward, C., 2009. A critical review of the empirical literature on the relation between anxiety and puberty. Clin. Psychol. Rev. 29 (1), 1–23. https://doi.org/10.1016/j.cpr.2008.09.005.

Rice, F., Sellers, R., Hammerton, G., et al., 2017. Antecedents of new-onset major depressive disorder in children and adolescents at high familial risk. JAMA Psychiatry 74 (2), 153–160. https://doi.org/10.1001/jamapsychiatry.2016.3140.

Rolls, E.T., Huang, C.-C., Lin, C.-P., Feng, J., Joliot, M., 2020. Automated anatomical labelling atlas 3. Neuroimage 206, 116189. https://doi.org/10.1016/j.neuroimage.2019.116189.

Rosseel, Y., 2012. lavaan: an R package for structural equation modeling. J. Stat. Softw. 48 (2), 1–36. https://doi.org/10.18637/jss.v048.i02.

Rubinow, D.R., Schmidt, P.J., 2019. Sex differences and the neurobiology of affective disorders. Neuropsychopharmacology 44 (1), 111–128. https://doi.org/10.1038/s41386-018-0148-z.

Sarkey, S., Azcoitia, I., Garcia-Segura, L.M., Garcia-Ovejero, D., DonCarlos, L.L., 2008. Classical androgen receptors in non-classical sites in the brain. Horm. Behav. 53, 753–764.

Selleck, R.A., Zhang, W., Samberg, H.D., Padival, M., Rosenkranz, J.A., 2018. Limited prefrontal cortical regulation over the basolateral amygdala in adolescent rats, 17171-17171 Sci. Rep. 8 (1). https://doi.org/10.1038/s41598-018-35649-0.

Sequeira, S.L., Rosen, D.K., Silk, J.S., Hutchinson, E., Allen, K.B., Jones, N.P., Price, R.B., Ladouceur, C.D., 2021. "Don't judge me!": Links between in vivo attention bias toward a potentially critical judge and fronto-amygdala functional connectivity during rejection in adolescent girls. Dev. Cogn. Neurosci. 49, 100960 https://doi.org/10.1016/j.dcn.2021.100960.

Shirtcliff, E.A., Ruttle, P.L., 2010. Immunological and neuroendocrine dysregulation following early deprivation and stress. In: Brisch, H. (Ed.), Attachment and Early Disorders of Development. Klett-Cotta & Stuttgart.

Shirtcliff, E.A., Dahl, R.E., Pollak, S.D., 2009. Pubertal development: correspondence between hormonal and physical development. Child Abus. Negl. 80 (2), 327–337.

Silvers, J.A., Insel, C., Powers, A., Franz, P., Helion, C., Martin, R.E., Weber, J., Mischel, W., Casey, B.J., Ochsner, K.N., 2017. vlPFC-vmPFC-amygdala interactions underlie age-related differences in cognitive regulation of emotion. Cereb. Cortex 27 (7), 3502–3514. https://doi.org/10.1093/cercor/bhw073.

Simerly, R.B., Chang, C., Muramatsu, M., Swanson, L.W., 1990. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridation study. J. Comp. Neurol. 294, 76–95.

Sisk, C.L., Foster, D.L., 2004. The neural basis of puberty and adolescence. Nat. Neurosci. 7, 1040–1047.

Sisk, C.L., Zehr, J.L., 2005. Pubertal hormones organize the adolescent brain and behavior. Front. Neuroendocrinol. 26, 163–174.

Smith, D.V., Hayden, B.Y., Truong, T.K., Song, A.W., Platt, M.L., Huettel, S.A., 2010. Distinct value signals in anterior and posterior ventromedial prefrontal cortex. J. Neurosci. 30 (7), 2490–2495. https://doi.org/10.1523/jneurosci.3319-09.2010.

Smith, S.M., Fox, P.T., Miller, K.L., Glahn, D.C., Fox, P.M., Mackay, C.E., Filippini, N., Watkins, K.E., Toro, R., Laird, A.R., Beckmann, C.F., 2009. Correspondence of the brain's functional architecture during activation and rest. Proc. Natl. Acad. Sci. USA 106 (31), 13040–13045. https://doi.org/10.1073/pnas.0905267106.

Somerville, L.H., Fani, N., McClure-Tone, E.B., 2011. Behavioral and neural representation of emotional facial expressions across the lifespan. Dev. Neuropsychol. 36 (4), 408–428. https://doi.org/10.1080/87565641.2010.549865.

Spielberg, J.M., Forbes, E.E., Ladouceur, C.D., Worthman, C.M., Olino, T.M., Ryan, N.D., Dahl, R.E., 2015. Pubertal testosterone influences threat-related amygdala-orbitofrontal cortex coupling. Soc. Cogn. Affect. Neurosci. 10 (3), 408–415.

Spielberg, J.M., Schwarz, J.M., Matyi, M.A., 2019. Anxiety in transition: neuroendocrine mechanisms supporting the development of anxiety pathology in adolescence and young adulthood. Front Neuroendocr. 55, 100791 https://doi.org/10.1016/j.yfrne.2019.100791.

Strawn, J.R., Bitter, S.M., Weber, W.A., Chu, W.J., Whitse, l R.M., Adler, C., Cerullo, M. A., Eliassen, J., Strakowski, S.M., DelBello, M.P., 2012. Neurocircuitry of generalized anxiety disorder in adolescents: a pilot functional neuroimaging and functional connectivity study. Depress Anxiety 29 (11), 939–947. https://doi.org/10.1002/da.21961.

Suzuki, Y., Tanaka, S.C., 2021. Functions of the ventromedial prefrontal cortex in emotion regulation under stress. Sci. Rep. 11 (1), 18225. https://doi.org/10.1038/s41598-021-97751-0.

Swartz, J.R., Carrasco, M., Wiggins, J.L., Thomason, M.E., Monk, C.S., 2014. Age-related changes in the structure and function of prefrontal cortex-amygdala circuitry in children and adolescents: a multi-modal imaging approach. Neuroimage 86, 212–220. https://doi.org/10.1016/j.neuroimage.2013.08.018.

Team, R.C., 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing (In). ⟨https://www.R-project.org/⟩.

Thijssen, S., Collins, P.F., Luciana, M., 2022. Does pubertal stage mediate the association between family environment and structure and function of the amygdala-mPFC circuit? a replication study of the longitudinal ABCD cohort. Dev. Cogn. Neurosci. 56, 101120 https://doi.org/10.1016/j.dcn.2022.101120.

Urry, H., van Reekum, C., Johnstone, T., Kalin, N., Thurow, M., Schaefer, H., Jackson, C., Frye, C., Greischar, L., Alexander, A., Davidson, R., 2006. Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. J. Neurosci. 26 (16), 4415–4425.

Van Wingen, G.A., Zylicz, S.A., Pieters, S., Mattern, C., Verkes, R.J., Buitelaar, J.K., Fernandez, G., 2010. Testosterone reduces amygdala-orbitofrontal cortex coupling. Psychoneuroendocrinology 35, 105–113.

de Vries, A.L., Doreleijers, T.A., Steensma, T.D., Cohen-Kettenis, P.T., 2011. Psychiatric comorbidity in gender dysphoric adolescents. J. Child Psychol. Psychiatry 52 (11), 1195–1202. https://doi.org/10.1111/j.1469-7610.2011.02426.x.

Wechsler, D., 1999. Wechsler abbreviated scale of intelligence. Psychol. Corp.

Windle, M., Lemer, R.M., 1986. The goodness of fit model of temperament-context relations: Interaction or correlation? In: Lerner, R.M., Lerner, J.V. (Eds.), New directions in child development, Temperament and psychosocial interaction in infancy and childhood. Jossey-Bass.

van Wingen, G.A., Zylicz, S.A., Pieters, S., Mattern, C., Verkes, R.J., Buitelaar, J.K., Fernández, G., 2009. Testosterone increases amygdala reactivity in middle-aged women to a young adulthood level. Neuropsychopharmacology 34 (3), 539–547. https://doi.org/10.1038/npp.2008.2.

Wu, M., Kujawa, A., Lu, L.H., Fitzgerald, D.A., Klumpp, H., Fitzgerald, K.D., Monk, C.S., Phan, K.L., 2016. Age-related changes in amygdala-frontal connectivity during emotional face processing from childhood into young adulthood. Hum. Brain Mapp. 37 (5), 1684–1695. https://doi.org/10.1002/hbm.23129.