Cerebral Cortex, 2025, **35**, bhaf218
https://doi.org/10.1093/cercor/bhaf218
Advance access publication date 11 August 2025
Original Article

Sex and age differences on how testosterone relates to brain activity during working memory among adolescents and young adults

Attakias T. Mertens[1], Katrina Myers[1], Delaney Sherman[1], Cecile D. Ladouceur[2], and Gaelle E. Doucet[1,3,4,*]

[1]Institute for Human Neuroscience, Boys Town National Research Hospital, 14090 Mother Teresa Lane, Boys Town, NE 68010, United States
[2]Department of Psychiatry, University of Pittsburgh School of Medicine, 3811 O'Hara Street, Pittsburgh, PA 15213, United States
[3]Center for Pediatric Brain Health, Boys Town National Research Hospital, 14090 Mother Teresa Lane, Boys Town, NE 68010, United States
[4]Department of Pharmacology and Neuroscience, Creighton University School of Medicine, 2500 California Plaza, Omaha 68178, NE United States
*Corresponding author: Gaelle E. Doucet, Institute for Human Neuroscience, Boys Town National Research Hospital, 14090 Mother Teresa Lane, 68010 Boys Town, NE, USA.
Email: gaelle.doucet@boystown.org

Working memory is an important cognitive process that develops throughout early life. During adolescence, there is marked improvement in this process that is associated with structural and functional brain changes. These changes have been linked to age; however, endogenous testosterone is thought to regulate structural and functional changes in the brain during puberty, with differential influences across adolescence into early adulthood. Thus, testosterone may have a direct impact on brain activity that is modulated by age. The current study aimed to examine this using a working memory functional magnetic resonance imaging (fMRI) task in adolescents and young adults. Saliva samples collected prior to scanning were assayed for endogenous testosterone levels. One hundred and forty-five typically developing participants (74 female), aged 12–25 yr, completed a working memory fMRI task. Results showed that, for the most difficult versus the 0back conditions, younger female participants (≤19) only had more deactivation in the anterior cingulate cortex with higher level of testosterone. In contrast, male participants showed increased activation in the precentral gyrus with higher testosterone, regardless of age. These findings indicate sex differences in how endogenous testosterone relates to the activity of different brain regions recruited during working memory. Furthermore, these associations vary across typical adolescent development.

Keywords: adolescence; development; fMRI; testosterone; working memory.

Introduction

Working memory (WM) is an executive function responsible for the processing and manipulation of information (Baddeley 1992). WM performance is predictive of individual differences in learning capabilities and future academic achievements in youth (Swanson 2004; Alloway and Alloway 2010). Previous research also suggests that WM is required for the development of skills in reasoning, problem solving, and reading comprehension (Baddeley 1992; Swanson 2004; Barrouillet et al. 2008; Arrington et al. 2014). While WM capacity continues to develop into early adulthood, it undergoes marked improvement during adolescence (Andre et al. 2015; Ferguson et al. 2021). Functional magnetic resonance imaging (fMRI) research has identified a network of brain regions that are active during WM tasks (Kwon et al. 2002; Andre et al. 2015; Rosenberg et al. 2020). Predominantly located in the lateral frontoparietal areas, these regions comprise the aptly named working memory network (WMN; Andre et al. 2015). Studies on WM development have found that adolescents show differences in patterns of neural activity within the WMN, compared to both children and young adults (Schweinsburg et al. 2005; Andre et al. 2015; Yang et al. 2015). Broadly, these include increased activation in frontoparietal regions with age until early adulthood (Kwon et al. 2002; Sridharan et al. 2008; Andre et al. 2015; Rosenberg et al. 2020).

Research has largely used age as a metric for developmental progression. Yet, other maturational processes that influence neurodevelopment, such as those driving puberty, are known to vary by age and sex (Bramen et al. 2011; Braams et al. 2015; Curtis et al. 2024). The onset of puberty is characterized by a rise in androgens (testosterone and dehydroepiandrosterone [DHEA]) and estrogens that underpin changes in physical characteristics (eg body hair, height) as well as motivated behavior and socio-affective processing (Op de Macks et al. 2016; Harden et al. 2018; Vijayakumar et al. 2019). For the current study, we focused specifically on testosterone, due to its role in brain development for both sexes. Indeed, testosterone relates to changes in both the structure and function of various frontoparietal regions involved in executive functioning. Adolescence is a pivotal time for cortical thinning within these regions. Testosterone has been found to differentially relate to this process, depending on both age and sex (Nguyen et al. 2013). Female adolescents tend to experience

Received: June 6, 2025. **Revised:** July 11, 2025. **Accepted:** July 14, 2025

such thinning earlier than their male counterparts; however, adolescent males show faster rates of thinning (Raznahan et al. 2010). In addition to these differences, female individuals show positive associations between their testosterone levels and cortical thickness for these frontoparietal regions prior to the onset of puberty. Post puberty, these associations become negative, similar to the pattern seen in their male counterparts across pubertal status (Nguyen et al. 2013).

Neurodevelopmental research on the influence of testosterone on brain function has largely focused on motivated behaviors and emotional processing (Op de Macks et al. 2016; Vijayakumar et al. 2019; White et al. 2020). Findings from neuroimaging studies on reward processing indicate that elevated testosterone levels are associated with reward-related striatal activation in longitudinal (Braams et al. 2015) and cross-sectional (Op de Macks et al. 2011) studies with adolescents. Elevated testosterone levels also correlate with stronger functional connectivity between striatal and frontal regions during a reward cue task, but only for female early adolescents (Ladouceur et al. 2019). Others have documented the influence of testosterone on the maturation of circuitry underlying emotion regulation during the transition from middle adolescence into young adulthood. Tyborowska et al. (2024) reported longitudinal changes of testosterone's impact on brain activation in the anterior prefrontal cortex (PFC) during incongruent vs. congruent trials on an emotion approach-avoidance task. Specifically, the positive effect of testosterone on PFC engagement decreased in adolescence (age 14–17) and became negative once participants were 20 yr old. This indicates a shift in the impact of testosterone across adolescence into adulthood from positive to negative relationships in regions relevant to emotion control. These findings are consistent with other developmental studies that report negative relationships between pubertal testosterone levels and prefrontal-amygdala functional connectivity during emotion processing (Spielberg et al. 2015) and resting state (Ladouceur et al. 2023), particularly in female youth. Together these findings indicate that testosterone directly influences the functioning of brain networks implicated in the regulation of emotion and behavior, which change with age.

Overall, previous literature has demonstrated that testosterone impacts brain activity during complex cognitive processes; however, it has largely focused on socioemotional processing. Despite testosterone impacting the structural development of brain regions responsible for executive functioning, its effect on brain activity during such processes, including WM, has received less investigation. Using fMRI, Alarcón et al. (2014) reported that testosterone levels were not able to explain sex differences in task activation during a spatial WM task. In contrast, a recent study using magnetoencephalography found differential effects of testosterone by sex on alpha and theta oscillations during encoding phases of a verbal WM task (Killanin et al. 2024). Additionally, in the maintenance phase of the task, higher alpha was related with higher testosterone, regardless of sex. Despite these studies, the full impact of testosterone on WM-related brain function across adolescent development remains unclear.

The current study aimed to address this gap in knowledge, potentially providing new insights into the physiological mechanisms of testosterone on brain activation during a WM task and to what extent these vary as a function of age and sex in adolescence into early adulthood. In a sample of 145 typically developing adolescents and young adults aged 12 to 25 yr old, we sought to determine (i) which brain regions were directly associated with testosterone during a WM fMRI task and (ii) to what extent these associations were moderated by age. Based on evidence linking testosterone to maturational structural changes in brain regions implicated in WM (Peper et al. 2009; Nguyen et al. 2013; Herting and Sowell 2016; Tamnes et al. 2017; Bethlehem et al. 2022; Tyborowska et al. 2024), we hypothesized that activation of frontoparietal regions within the WMN would be associated with differences in testosterone levels. Specifically, we hypothesized that these associations would be stronger in younger participants than older participants, given the difference in variation in sex hormones during adolescence compared to adulthood (Tyborowska et al. 2024). Lastly, we expected different results in males and females, due to the significant difference in testosterone levels between the sexes from this age period throughout the lifespan. Additionally, considering the known sex differences regarding the effects of testosterone on the rate of structural brain changes (Peper et al. 2009; Raznahan et al. 2010; Herting and Sowell 2016; Tamnes et al. 2017; Bethlehem et al. 2022), we examined female and male participants separately. As there is currently little evidence of sex differences in testosterone-related maturational changes of brain activity for specific regions related to WM; we sought to investigate which regions showed significant effects of testosterone within each sex and examine if the sexes showed unique regions of effect.

Table 1. Participant demographics.

	Female	Male
N	74	71
Age (years)	19.09 (3.91)	19.08 (3.94)
FSIQ-4	111.70 (12.76)	114.44 (11.36)
Accuracy (%)		
2-back	88.6% (6.7%)	90.0% (7.3%)
0-back	97.5% (3.4%)	96.8% (4.4%)
Reaction times (ms)		
2-back	647.44 (82.04)	631.94 (84.98)
0-back	570.02 (103.56)	542.88 (108.48)

Note. Accuracies and reaction times are shown for 0-back and 2-back as the contrast of interest involves both conditions. Reaction times calculated from accurate trials only. There were no significant differences for any variable based on sex. *Mean* (*SD*) are reported.

Materials and methods

Participants

A total of 165 typically developing participants from ages 12 to 25 ($M = 19.09$, $SD = 3.97$), were recruited for this study (84 female, 81 male). Exclusion criteria included participants who did not meet safety criteria for the MRI scan (metal implants, pregnancy, etc), were using recreational or nonprescribed drugs, or had a diagnosis of a psychiatric or neurologic disorder. Data from 20 participants were not included in the analyses due to either: not completing the MRI scan ($n = 3$), having a maximum in-scanner motion greater than 3 mm ($n = 6$), not having hormone data ($n = 7$), or having task accuracy lower than 70% ($n = 4$). After applying these criteria, the total sample was 145 participants. Descriptive statistics including age, IQ scores, and task behavior within relevant conditions for each sex can be found in Table 1. The study was approved by the institutional review board (IRB # 21-17-XP) from Boys Town National Research Hospital and the participants and/or their legal guardian consented to participate in the research, while the children provided written assent.

N-back

The design for this task was adapted from Lytle et al. (2020). E-Prime 3 software was used to create and run all task stimuli

(Psychology Software Tools, Pittsburgh, PA). Prior to beginning this task, a practice session with a brief version of the task was completed to ensure participants understood the instructions. Participants completed two runs of an N-back task during an fMRI scan, which included two domains of WM (ie verbal and spatial versions; Lytle et al. 2020). These versions were analyzed in conjunction to obtain an encompassing assessment of the different facets of WM. Stimuli for this task included a centered fixation cross and individual letters that could appear in one of the four corners around the cross. Participants were told to enter their responses using buttons that represented a "match" (index finger) or "no match" (middle finger). Each run contained the same stimuli (same letters across the runs) but differed in the instructions to focus on either verbal (letter itself) or spatial (location) aspects. When completing the verbal run, participants were told to focus on the specific letter shown and ignore its location. In contrast, for the spatial run, they were instructed to focus on the location of the letter and ignore the letter itself. Runs included three conditions: 0-back, 1-back, and 2-back. For the 0-back conditions, participants had to answer if the letter "x" was presented during the verbal run and if a letter was above the fixation cross in the spatial. These conditions were presented in a block design. Each run consisted of 12 blocks: four for each condition. Individual blocks included 12 trials, of which only four were target trials. Prior to the start of each block, instructions indicating the condition were presented to participants for 2,000 ms. Within individual trials, letters were presented for 1,200 ms, followed by a fixation period of 800 ms. The order of target/non-target trials in a block and blocks within a run were pseudo-randomized, and the run order was counter-balanced across participants.

Saliva collections and testosterone quantification

Saliva samples were collected prior to the MRI scan. Participants were told to refrain from eating or drinking for at least an hour prior to the collection. Participants were instructed to passively drool into a collection tube until saliva exceeded the fill line marked on the tube, which indicated 2.0 mL. Samples were stored in a freezer at –20°C. Saliva was tested at the Institute for Interdisciplinary Salivary Bioscience Research at UC Irvine, in duplicate using a commercially available and well-validated salivary enzyme-immunoassays kits (www.salimetrics.com). Assay range was 6.1 to 600 pg/mL, with a sensitivity at 1 pg/mL.

Samples from each participant were assayed in the same plate to minimize variation across plates. The average intra-assay coefficient of variation was 4.57 ($SD = 2.23$) and the inter-assay coefficient average was 9.38 ($SD = 1.29$). To further account for any variation in hormone values across plates, assay results (ie testosterone values) were run through ComBat Harmonization v1.0.1 for MATLAB (2023a). After this, participants' data were separated by sex for further analyses (Ladouceur et al. 2019). The *R* library *sur* was used to assess the distribution characteristics of testosterone (Harel 2020). Testosterone was positively skewed (skewness = 1.43, SE = 0.20). Therefore, testosterone levels for each sex were winsorized within 2.5 standard deviations of their respective averages using the *quest* library (Disabato 2023), after which they were log-transformed, in line with previous research (Dismukes et al. 2015; Ladouceur et al. 2019; Ladouceur et al. 2023). Finally, the time of sample collection was accounted for by regressing it on the transformed hormone values and saving the standardized residuals. These will be referred to as testosterone levels throughout the following sections.

MRI data acquisition and preprocessing

Scans were acquired using a 3T Siemens Prisma scanner with 32-channel head coils. Structural images were collected using a T1-weighted, 3D magnetization-prepared rapid gradient-echo sequence with the following parameters: repetition time/echo time (TR/TE) = 2,400/2.05 ms, field of view (FOV) = 256×256 mm, 1 mm isotropic resolution, inversion time (TI) = 1,000 ms, flip angle = 8°, bandwidth = 240 Hz/Pixel, scan time ~ 8 min. Two functional scans of the N-back task were collected using a multi-band T2* sequence with the following parameters: TR/TE = 480/29.2 ms, flip angle = 44°, voxel size = 3×3×3 mm, echo spacing = 0.51 ms, bandwidth = 2,770 Hz/Pixel, number of axial slices = 56, multi-band acceleration factor = 8. A total of 702 volumes were collected for each functional run. FMRI data was preprocessed using the standard pipeline from fMRIPrep v23.0.2 (Esteban et al. 2017; Esteban et al. 2019), including slice timing, correcting for head motion, coregistration to T1w, and normalization to Montreal Neurological Institute (MNI) space. Head motion estimates were calculated during the correction step and saved to a confounds file. Lastly, smoothing was conducted in SPM12 with a Gaussian kernel of 6×6×6 mm full-width half-maximum.

Statistical analyses

Activation cluster identification

First, we aimed to identify brain clusters that were significantly associated with testosterone levels during the N-back task, using the *2-back–0-back* contrast, within sex. General linear model analyses were run in SPM12. The first level analyzed individual participants' images across both runs and included six conditions of interest (0-, 1- and 2-back for each run) and six head motion regressors of no interest for each run. The contrast of focus included the combined comparison of the most difficult condition, 2-back, versus 0-back, combining both runs. Participants were divided by sex for the second-level group analyses. A one-sample t-test was run using the 2-back versus 0-back contrast, with testosterone levels as a covariate of interest. The significance threshold was set at $P < 0.001$ (uncorrected, whole-brain level) and $P < 0.05$ (FWE corrected at the cluster level, reflecting $k > \sim 50$).

Moderation analyses

Next, we aimed to determine to what extent this relationship between testosterone and brain activation was moderated by age. For this, R Statistical Software (v4.4.0; R Core Team 2021) was used to assess potential moderation effects, inside RStudio (RStudio Team 2022). All variables were mean centered prior to the following tests. Moderation analyses were run using PROCESS Macro for R (Hayes 2022). These models assessed the impact of age on the relationship between testosterone (predictor) and cluster activation during the N-back task (DV). Models were resampled 5,000 times with bootstrapping. Significant moderations underwent simple slopes testing on the *mean*, +1 *SD*, and –1 *SD* of age to determine the strength and significance of the effect of testosterone on cluster activation across these ages. Robust standard errors were used to find confidence intervals for the simple slopes. If simple slopes analyses indicated that testosterone did not significantly affect cluster activation across all ages, the Johnson–Neyman technique was applied to identify exact regions of significance along the continuous moderator of age, while controlling for false discovery rate (FDR; Bauer and Curran 2005; Esarey and Sumner 2018; Johnson and Neyman 1936; Rast et al. 2014). Simple slopes testing, interaction plots, and the

Table 2. Moderation analysis statistics for age, testosterone, and interaction.

	β	SE	t	p	LLCI	ULCI
Testosterone	**−0.52**	**0.05**	**−5.48**	**<0.001**	**−0.33**	**−0.16**
Age	−0.07	0.01	−0.78	0.437	−0.03	0.01
Interaction	**0.27**	**0.01**	**2.87**	**0.006**	**0.01**	**0.06**

Note. Dependent variable is anterior cingulate activity. LLCI = Lower Limit Confidence Interval. ULCI = Upper Limit Confidence Interval. Bold refers to the significant variables ($P < 0.05$).

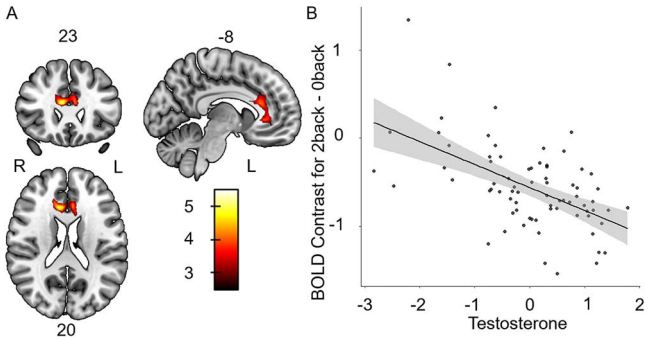

Fig. 1. *Task activation and association with testosterone: female participants. Note.* A) Cluster in the anterior cingulate region significantly associated with testosterone from the 2-back vs. 0-back contrast. B) Association between testosterone levels and deactivation in the anterior cingulate. Scores on testosterone are standardized regression residuals and thus have no units. The testosterone measurement used in the regression included logged pg/mL.

Johnson–Nehman technique were conducted using the *interactions* library (Long 2024). Lastly, the *lm.beta* library was used to calculate standardized regression coefficients (Behrendt 2023).

Results

Behavioral results

There were no sex differences in age ($t = 0.02$ $P = 0.99$), task accuracy (2-back: $t = -1.18$, $P = 0.24$; 0-back: $t = 1.03$, $P = 0.31$), or reaction times from correct trials (2-back: $t = 1.12$, $P = 0.27$; 0-back: $t = 1.54$, $P = 0.13$, Table 1). Testosterone levels were not significantly associated with age for female participants ($r = 0.09$, $P = 0.43$), but were significantly, positively correlated with age in male participants ($r = 0.52$, $P < 0.001$).

Brain results: female participants

Whole-brain analyses revealed one cluster that was significantly associated with testosterone, such that a region located in the anterior cingulate cortex (ACC) had stronger deactivation at higher levels of testosterone ($p_{FWE} = 1.79 \times 10^{-9}$, $k_E = 475$; Fig. 1). Moderation analysis testing for the effect of age on the relationship between testosterone levels and ACC cluster activity indicated a significant moderation effect ($F(3,70) = 16.17$, $P < 0.001$, $R^2 = 0.41$; Table 2). Model results showed that the interaction between age and testosterone was significant ($\Delta R^2 = 0.07$, $F(1,70) = 8.22$, $P = 0.006$, $f^2 = 0.12$; Table 2) and that this interaction alone accounted for 7% of the variance in ACC cluster activity. Simple slopes testing indicated that testosterone was a significant, negative predictor for activity in the ACC cluster for individuals at the average age of the sample (19.09 yr) and one *SD* younger (15.18 yr), but not for those one *SD* older than the average (23 yr; Fig. 2; Table 3). The Johnson–Nehman technique found that the slope of testosterone was significant ($p_{FDR} < 0.05$) specifically for participants between the ages of 12.27 to 21.58 yr.

Brain results: male participants

The whole brain analysis revealed one significant cluster associated with testosterone level, in the male participants. The cluster was in the right precentral gyrus ($p_{FWE} = 0.015$, $k_E = 80$) and had a positive association with testosterone (Fig. 3). The moderation model was found to be significant, $F(3,67) = 8.87$, $P < 0.001$, $R^2 = 0.28$. While testosterone was a significant, positive predictor of activation in the precentral cluster, the interaction between age and testosterone was not significant $F(1,67) = 0.30$, $P = 0.58$ (Table 4), suggesting that age did not have an impact on this relationship. Due to this, the interaction was not tested further.

Discussion

Current findings indicate sex differences in how testosterone levels relate to WM function. While not within the WMN, the regions found to significantly relate to testosterone within each sex belong to networks/systems related to WM functioning. In regard to the effect of age, the findings partially support our hypothesis that testosterone effects would be stronger in younger participants. The moderation in female participants showed that younger participants had a stronger association between testosterone and activity in the ACC. In contrast, results from the moderation for male participants showed no significant effect of age, indicating activity in the right precentral gyrus was related to testosterone regardless of age.

Our main findings showed that, for female participants, higher testosterone was associated with stronger deactivation in the ACC during the most difficult WM condition compared to the 0back condition. The ACC plays an important role in maintaining goal-directed behavior, especially regarding decision-making and adjusting responses/actions (Rolls 2019). Prior research has suggested that the ACC is involved in attention shifting and cognitive control processes, due to information it receives from cortical and subcortical regions (Bush et al. 2000; Eshel et al. 2007; Holroyd and Yeung 2012; Shenhav et al. 2013; Rolls 2019; Salehinejad et al. 2021). The ACC is part of the default mode network (DMN), which, in line with the current findings, is often deactivated during difficult cognitive tasks (Shulman et al. 1997; Mazoyer et al. 2001; Raichle et al. 2001; Lawrence et al. 2003; Buckner et al. 2008; Ye et al. 2022). Greater deactivation in the DMN occurs as individuals approach adulthood and is associated with more efficient cognitive functioning (Buckner et al. 2008; Andrews-Hanna et al. 2010; Raichle 2015; Sandhu et al. 2020; Roig-Herrero et al. 2022).

Our findings are partially consistent with those from Alarcón and colleagues. Although Alarcón et al. (2014) did not find a significant effect of testosterone on WM, they did report sex differences. Particularly, they reported that female, but not male adolescents, showed greater deactivation in the DMN, including

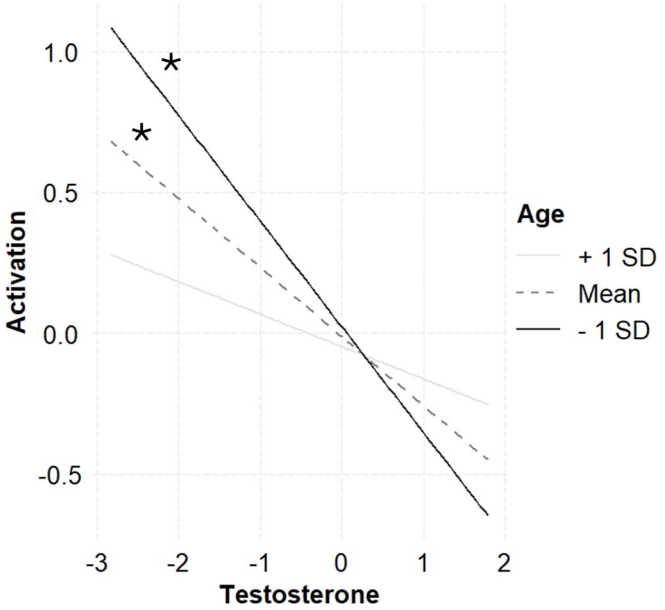

Fig. 2. *Simple slopes interaction plot: female participants. Note.* Figure shows the slopes for the association between testosterone levels and anterior cingulate activity in female participants at the mean age (19.09 yr), one standard deviation above the mean (23 yr), and one standard deviation below the mean (15.18 yr). SD = standard deviation. $^{+}$ indicates slopes of $p_{FDR} < 0.05$. The testosterone measurement used included logged pg/mL.

Table 3. Simple slopes for testosterone at different ages: females participants.

	Estimate	SE	*t*	*p*
SD below	**−0.38**	**0.13**	**−2.81**	**0.006**
Mean	**−0.25**	**0.07**	**−3.74**	**<0.001**
SD above	−0.12	0.10	−1.14	0.260

Note. SD below = Slope of Testosterone when Age = −1 SD. Mean = Slope of Testosterone when Age = mean. SD Above = Slope of Testosterone when Age = +1 SD.

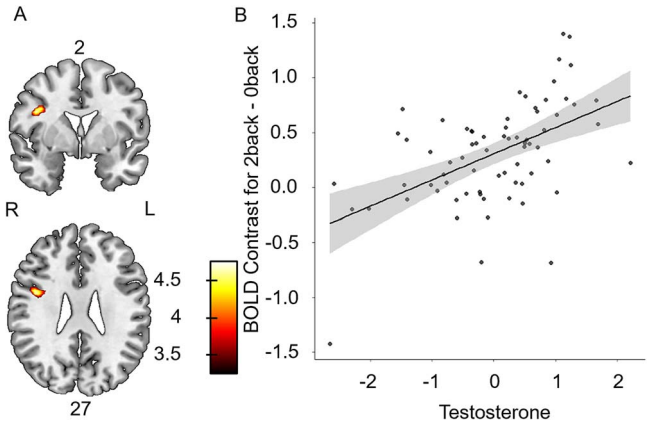

Fig. 3. *Task activation and association with testosterone in male participants. Note.* A) Cluster in the right precentral gyrus region significantly associated with testosterone from the 2-back vs. 0-back contrast. B) Positive linear association between testosterone levels and activation in cluster. The testosterone measurement used in the regression included logged pg/mL.

the ACC, during a WM task. Our current work further expands on these findings by suggesting this increased deactivation is related to testosterone levels in female participants at and under the age of 21.58. In contrast, testosterone levels in adult female participants from the current sample were not related to ACC activity during the WM task. These effects are further emphasized by the lack of association between age and testosterone in female participants. This result was unsurprising as testosterone levels vary less in females than males during adolescence and have been found to reach a plateau, where levels stop significantly increasing, around 14 yr (Senefeld et al. 2020). Taken together, these results imply that, despite testosterone levels not significantly changing in females between the ages of 12 and 25, the hormone did significantly affect brain function for the female participants 21.58 yr old and younger. Thus, this age range appears to be an important developmental period for the effects of testosterone in this context within female adolescents and young adults. Additionally, this highlights that the driving point behind these effects may lie in a developmental time window, rather than being spurred by an increase in testosterone. This further demonstrates the need for future work to examine the direct effects of testosterone on brain activity and how they may be impacted by age, as analyses controlling or accounting for age in this context would be unable to elucidate such results.

Prior work has also found changes in testosterone effects on brain activation to occur around late adolescence/early adulthood (Tyborowska et al. 2024). Tyborowska et al. (2024) found that, during an emotion approach-avoidance task, participants ages 14 to 17 had a positive association between testosterone and activity in the lateral anterior PFC; which ultimately turned negative at age 20 (Tyborowska et al. 2024). Although these findings were not in the context of WM, the current study aligns with these previous results in finding that age modulates the association between brain activity and testosterone during late adolescence/early adulthood. While the current findings did not find a shift in valence of the effects of testosterone with age, they

Table 4. Moderation regression statistics: male participants.

	β	SE	t	p	LLCI	ULCI
Testosterone	**0.41**	**0.06**	**3.28**	**0.002**	**0.08**	**0.31**
Age	0.15	0.01	1.27	0.208	−0.01	0.05
Interaction	−0.06	0.01	−0.55	0.584	−0.03	0.02

Note. Dependent variable is right precentral gyrus activity. LLCI = Lower Limit Confidence Interval. ULCI = Upper Limit Confidence Interval. Bold refers to the significant variables ($P < 0.05$).

do show significant associations for younger participants that are not significant for the oldest participants.

Regarding male participants, higher testosterone levels were associated with stronger activation in the right precentral gyrus during the most difficult WM condition compared to the 0back; however, this relationship was not modulated by age. Evidence of the right precentral gyrus' role in WM is limited; however, some previous studies have suggested that it belongs to a larger network involved in cognitive and motor aspects of inhibitory behaviors (Bhattacharjee et al. 2020; Stein et al. 2021). In support of this, prior studies have found the right precentral gyrus to be associated with response inhibition and even WM tasks (Moon et al. 2016; Ren et al. 2019; Bhattacharjee et al. 2020; Stein et al. 2021; Pawlak et al. 2022). While the absence of an age effect was unexpected, testosterone levels in male adolescents can vary widely during this developmental period (Vadakkadath Meethal and Atwood 2005; Khairullah et al. 2014) and continue to increase through early adulthood as seen in the current study.

While the exact mechanisms through which pubertal hormones impact WM functional development are not yet fully understood. Despite this, prior research does present one possible method for testosterone specifically: through impacting dopamine (DA) signaling. DA in the PFC, a major region involved in executive functioning, modulates WM performance (Floresco 2013). Evidence from animal studies demonstrates that testosterone and other androgens affect DA synthesis, receptors (expression, dimerization, and binding affinity), release, and extracellular levels (Seib et al. 2023). Rodent studies have directly documented the impact of testosterone on WM performance via the regulation of DA synthesis enzymes in the PFC (Seib et al. 2023). In relation to regions from the current study's findings, although fMRI does not yield information about D1 receptors, other studies have found that D1 receptors in the ACC relate to executive function performance. Mainly, the activity of DA receptors within the ACC has been found to influence decision-making processes (Schweimer and Hauber 2006). Regarding the right precentral gyrus, there is less evidence related to executive functioning; however, one study found a negative relationship between DA receptor availability in this region and reaction time during a stop signal task (Albrecht et al. 2014).

Findings from this study should be interpreted in light of some limitations. First, we used a cross-sectional design, which precludes documenting developmental changes in testosterone and WM within individuals. Additionally, this study only focused on functional aspects of WM impacted by testosterone, with no connections to any structural brain measures. Future neurodevelopmental studies should implement longitudinal designs aimed at assessing structural and functional brain aspects across multiple cognitive domains as well as other pubertal hormones such as DHEA or estradiol. The age range used in this study was chosen to capture the effects of testosterone across adolescence into young adulthood. Pubertal onset is variable across individuals, with studies suggesting it can start as early as 8 yr old in females and 9 yr old in males (Howard and Dunkel 2019). The results from this study suggested that testosterone had a significant effect on brain activity for the youngest participants, both females and males, around the age of 12. Thus, future studies may benefit from including earlier ages to determine when these testosterone effects begin. As prior work has found testosterone to impact the structural development of regions within the WMN (Raznahan et al. 2010; Nguyen et al. 2013), activity in such regions was initially hypothesized to show associations with hormone levels in the current study. Although regions outside the WMN were found in the current study, future research should continue investigating testosterone's potential impact on the functional, developmental changes of frontoparietal regions. Outside of WM, these regions are also involved with other executive functions that may be more impacted by testosterone. Lastly, the significant moderation in this study ended up with a small effect size.

In sum, the current study documents sex differences in the relationship between testosterone levels and brain activation during performance of a WM task among adolescents and young adults. Furthermore, the results underscore the need for future mechanistic studies in order to understand sex differences in how pubertal hormones impact the neurodevelopment of WM processes in adolescence and early adulthood.

Author contributions

Attakias Mertens (Conceptualization, Formal analysis, Methodology, Visualization, Writing—original draft), Katrina Myers (Investigation), Delaney Sherman (Formal analysis), Cecile Ladouceur (Validation, Writing—review & editing), and Gaelle Doucet (Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing—review & editing).

Funding

This work was supported by the National Institute for Health (P20GM144641).

Conflict of interest statement. None declared.

Data availability

The deidentified data and scripts used in this article are available on request to the corresponding author.

References

Alarcón G, Cservenka A, Fair DA, Nagel BJ. 2014. Sex differences in the neural substrates of spatial working memory during adolescence are not mediated by endogenous testosterone. *Brain Res*. 1593: 40–54. https://doi.org/10.1016/j.brainres.2014.09.057.

Albrecht DS, Kareken DA, Christian BT, Dzemidzic M, Yoder KK. 2014. Cortical dopamine release during a behavioral response inhibition task. *Synapse*. 68:266–274. https://doi.org/10.1002/syn.21736.

Alloway TP, Alloway RG. 2010. Investigating the predictive roles of working memory and IQ in academic attainment. *J Exp Child Psychol*. 106:20–29. https://doi.org/10.1016/j.jecp.2009.11.003.

Andre J, Picchioni M, Zhang R, Toulopoulou T. 2015. Working memory circuit as a function of increasing age in healthy adolescence: a systematic review and meta-analyses. *NeuroImage : Clinical*. 12: 940–948. https://doi.org/10.1016/j.nicl.2015.12.002.

Andrews-Hanna JR, Reidler JS, Sepulcre J, Poulin R, Buckner RL. 2010. Functional-anatomic fractionation of the Brain's default network. *Neuron*. 65:550–562. https://doi.org/10.1016/j.neuron.2010.02.005.

Arrington CN, Kulesz PA, Francis DJ, Fletcher JM, Barnes MA. 2014. The contribution of attentional control and working memory to reading comprehension and decoding. *Scientific Studies of Reading : The Official Journal of the Society for the Scientific Study of Reading*. 18: 325–346. https://doi.org/10.1080/10888438.2014.902461.

Baddeley A. 1992. Working memory. *Science*. 255:556–559. https://doi.org/10.1126/science.1736359.

Barrouillet P, Lépine R, Camos V. 2008. Is the influence of working memory capacity on high-level cognition mediated by complexity or resource-dependent elementary processes? *Psychon Bull Rev*. 15:528–534. https://doi.org/10.3758/PBR.15.3.528.

Bauer DJ, Curran PJ. 2005. Probing interactions in fixed and multilevel regression: inferential and graphical techniques. *Multivar Behav Res*. 40:373–400. https://doi.org/10.1207/s15327906mbr4003_5.

Behrendt S. 2023. *Lm.Beta: add standardized regression coefficients to linear-model-objects (version 1.7–2) [computer software], Comprehensive R Archive Network (CRAN)*. https://cran.r-project.org/package=lm.beta.

Bethlehem RAI et al. 2022. Brain charts for the human lifespan. *Nature*. 604:525–533. https://doi.org/10.1038/s41586-022-04554-y.

Bhattacharjee S et al. 2020. The role of primary motor cortex: more than movement execution. *J Mot Behav*. 53:258–274. https://doi.org/10.1080/00222895.2020.1738992.

Braams BR, van Duijvenvoorde ACK, Peper JS, Crone EA. 2015. Longitudinal changes in adolescent risk-taking: a comprehensive study of neural responses to rewards, pubertal development, and risk-taking behavior. *J Neurosci*. 35:7226–7238. https://doi.org/10.1523/JNEUROSCI.4764-14.2015.

Bramen JE et al. 2011. Puberty influences medial temporal lobe and cortical Gray matter maturation differently in boys than girls matched for sexual maturity. *Cerebral Cortex (New York, NY)*. 21: 636–646. https://doi.org/10.1093/cercor/bhq137.

Buckner RL, Andrews-Hanna JR, Schacter DL. 2008. The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci*. 1124:1–38. https://doi.org/10.1196/annals.1440.011.

Bush G, Luu P, Posner MI. 2000. Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci*. 4:215–222. https://doi.org/10.1016/S1364-6613(00)01483-2.

Curtis M et al. 2024. Disentangling the unique contributions of age, pubertal stage, and pubertal hormones to brain structure in childhood and adolescence. *Dev Cogn Neurosci*. 70:101473. https://doi.org/10.1016/j.dcn.2024.101473.

Disabato, D. (2023). *Quest: prepare questionnaire data for analysis (version 0.2.0) [computer software] Comprehensive R Archive Network (CRAN)*. https://cran.r-project.org/web/packages/quest/index.html.

Dismukes AR, Shirtcliff EA, Hanson JL, Pollak SD. 2015. Context influences the interplay of endocrine axes across the day. *Dev Psychobiol*. 57:731–741. https://doi.org/10.1002/dev.21331.

Esarey J, Sumner JL. 2018. Marginal effects in interaction models: determining and controlling the false positive rate. *Comp Pol Stud*. 51:1144–1176. https://doi.org/10.1177/0010414017730080.

Eshel N, Nelson EE, Blair J, Pine DS, Ernst M. 2007. Neural substrates of choice selection in adults and adolescents: development of the ventrolateral prefrontal and anterior cingulate cortices. *Neuropsychologia*. 45:1270–1279. https://doi.org/10.1016/j.neuropsychologia.2006.10.004.

Esteban O et al. 2017. *Poldracklab/fmriprep: 1.0.0-rc5 (version 1.0.0-rc5) [computer software]*. Zenodo, 10.5281/zenodo.996169.

Esteban O et al. 2019. FMRIPrep: a robust preprocessing pipeline for functional MRI. *Nat Methods*. 16:111–116. https://doi.org/10.1038/s41592-018-0235-4.

Ferguson HJ, Brunsdon VEA, Bradford EEF. 2021. The developmental trajectories of executive function from adolescence to old age. *Sci Rep*. 11:1382. https://doi.org/10.1038/s41598-020-80866-1.

Floresco SB. 2013. Prefrontal dopamine and behavioral flexibility: shifting from an "inverted-U" toward a family of functions. *Front Neurosci*. 7:62. https://doi.org/10.3389/fnins.2013.00062.

Harden KP et al. 2018. Developmental differences in reward sensitivity and sensation seeking in adolescence: testing sex-specific associations with gonadal hormones and pubertal development. *J Pers Soc Psychol*. 115:161–178. https://doi.org/10.1037/pspp0000172.

Harel, D. (2020). *Companion to "statistics using R: an integrative approach" [R package Sur version 1.0.4]*. Comprehensive R Archive Network (CRAN). https://CRAN.R-project.org/package=sur.

Hayes, A. F. (2022). *Introduction to mediation, moderation, and conditional process analysis: a regression-based approach*. Guilford Publications. http://www.guilford.com/p/hayes3.

Herting MM, Sowell ER. 2016. Puberty and structural brain development in humans. *Front Neuroendocrinol*. 44:122–137. https://doi.org/10.1016/j.yfrne.2016.12.003.

Holroyd CB, Yeung N. 2012. Motivation of extended behaviors by anterior cingulate cortex. *Trends Cogn Sci*. 16:122–128. https://doi.org/10.1016/j.tics.2011.12.008.

Howard SR, Dunkel L. 2019. Delayed puberty—phenotypic diversity, molecular genetic mechanisms, and recent discoveries. *Endocr Rev*. 40:1285–1317. https://doi.org/10.1210/er.2018-00248.

Johnson PO, Neyman J. 1936. Tests of certain linear hypotheses and their application to some educational problems. *Statistical Research Memoirs*. 1:57–93.

Khairullah A et al. 2014. Testosterone trajectories and reference ranges in a large longitudinal sample of male adolescents. *PLoS One*. 9:e108838. https://doi.org/10.1371/journal.pone.0108838.

Killanin AD et al. 2024. Effects of endogenous testosterone on oscillatory activity during verbal working memory in youth. *Hum Brain Mapp*. 45:e26774. https://doi.org/10.1002/hbm.26774.

Kwon H, Reiss AL, Menon V. 2002. Neural basis of protracted developmental changes in visuo-spatial working memory. *Proc Natl Acad Sci USA*. 99:13336–13341. https://doi.org/10.1073/pnas.162486399.

Ladouceur CD et al. 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.

Ladouceur CD, Henry T, Ojha A, Shirtcliff EA, Silk JS. 2023. Fronto-amygdala resting state functional connectivity is associated with anxiety symptoms among adolescent girls more advanced in pubertal maturation. *Dev Cogn Neurosci*. 60:101236. https://doi.org/10.1016/j.dcn.2023.101236.

Lawrence NS, Ross TJ, Hoffmann R, Garavan H, Stein EA. 2003. Multiple neuronal networks mediate sustained attention. *J Cogn Neurosci*. 15:1028–1038. https://doi.org/10.1162/089892903770007416.

Long, J. A. (2024). *Interactions: comprehensive, user-friendly toolkit for probing interactions (version 1.2.0) [computer software] Comprehensive R Archive Network (CRAN)*. https://cran.r-project.org/web/packages/interactions/index.html.

Lytle MN, Hammer R, Booth JR. 2020. A neuroimaging dataset on working memory and reward processing in children with and without ADHD. *Data Brief*. 31:105801. https://doi.org/10.1016/j.dib.2020.105801.

Mazoyer B et al. 2001. Cortical networks for working memory and executive functions sustain the conscious resting state in man. *Brain Res Bull*. 54:287–298. https://doi.org/10.1016/s0361-9230(00)00437-8.

Moon CM, Sundaram T, Choi NG, Jeong GW. 2016. Working memory dysfunction associated with brain functional deficits and cellular metabolic changes in patients with generalized anxiety disorder. *Psychiatry Res Neuroimaging*. 254:137–144. https://doi.org/10.1016/j.pscychresns.2016.06.013.

Nguyen TV et al. 2013. Testosterone-related cortical maturation across childhood and adolescence. *Cerebral Cortex (New York, NY)*. 23:1424–1432. https://doi.org/10.1093/cercor/bhs125.

Op de Macks ZA et al. 2011. Testosterone levels correspond with increased ventral striatum activation in response to monetary rewards in adolescents. *Dev Cogn Neurosci*. 1:506–516. https://doi.org/10.1016/j.dcn.2011.06.003.

Op de Macks ZA et al. 2016. The effect of social rank feedback on risk taking and associated reward processes in adolescent girls. *Soc Cogn Affect Neurosci*. 12:240–250. https://doi.org/10.1093/scan/nsw125.

Pawlak M, Bray S, Kopala-Sibley DC. 2022. Resting state functional connectivity as a marker of internalizing disorder onset in high-risk youth. *Sci Rep*. 12:21337. https://doi.org/10.1038/s41598-022-25805-y.

Peper JS et al. 2009. Sex steroids and brain structure in pubertal boys and girls. *Psychoneuroendocrinology*. 34:332–342. https://doi.org/10.1016/j.psyneuen.2008.09.012.

R Core Team (2021). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Raichle ME. 2015. The brain's default mode network. *Annu Rev Neurosci*. 38:433–447. https://doi.org/10.1146/annurev-neuro-071013-014030.

Raichle ME et al. 2001. A default mode of brain function. *Proc Natl Acad Sci*. 98:676–682. https://doi.org/10.1073/pnas.98.2.676.

Rast P, Rush J, Piccinin A, Hofer SM. 2014. The identification of regions of significance in the effect of multimorbidity on depressive symptoms using longitudinal data: an application of the Johnson-Neyman technique. *Gerontology*. 60:274–281. https://doi.org/10.1159/000358757.

Raznahan A et al. 2010. Longitudinally mapping the influence of sex and androgen signaling on the dynamics of human cortical maturation in adolescence. *Proc Natl Acad Sci USA*. 107:16988–16993. https://doi.org/10.1073/pnas.1006025107.

Ren Z et al. 2019. The different brain mechanisms of object and spatial working memory: voxel-based morphometry and resting-state functional connectivity. *Front Hum Neurosci*. 13:248. https://doi.org/10.3389/fnhum.2019.00248.

Roig-Herrero A et al. 2022. Default mode network components and its relationship with anomalous self-experiences in schizophrenia: a rs-fMRI exploratory study. *Psychiatry Res Neuroimaging*. 324:111495. https://doi.org/10.1016/j.pscychresns.2022.111495.

Rolls E. 2019. The cingulate cortex and limbic systems for emotion, action, and memory. *Brain Struct Funct*. 224:3001–3018. https://doi.org/10.1007/s00429-019-01945-2.

Rosenberg MD et al. 2020. Behavioral and neural signatures of working memory in childhood. *J Neurosci*. 40:5090–5104. https://doi.org/10.1523/JNEUROSCI.2841-19.2020.

RStudio Team (2022). *RStudio: integrated development environment for R*. RStudio, PBC, Boston, MA http://www.rstudio.com/.

Salehinejad MA, Ghanavati E, Rashid MHA, Nitsche MA. 2021. Hot and cold executive functions in the brain: a prefrontal-cingular network. *Brain and Neuroscience Advances*. 5:23982128211007769. https://doi.org/10.1177/23982128211007769.

Sandhu Z et al. 2020. Parcellation-based anatomic modeling of the default mode network. *Brain Behav*. 11:e01976. https://doi.org/10.1002/brb3.1976.

Schweimer J, Hauber W. 2006. Dopamine D1 receptors in the anterior cingulate cortex regulate effort-based decision making. *Learn Mem*. 13:777–782. https://doi.org/10.1101/lm.409306.

Schweinsburg AD, Nagel BJ, Tapert SF. 2005. fMRI reveals alteration of spatial working memory networks across adolescence. *Journal of the International Neuropsychological Society: JINS*. 11:631–644. https://doi.org/10.1017/S1355617705050757.

Seib DR, Tobiansky DJ, Meitzen J, Floresco SB, Soma KK. 2023. Neurosteroids and the mesocorticolimbic system. *Neurosci Biobehav Rev*. 153:105356. https://doi.org/10.1016/j.neubiorev.2023.105356.

Senefeld JW et al. 2020. Divergence in timing and magnitude of testosterone levels between male and female youths. *JAMA*. 324:99–101. https://doi.org/10.1001/jama.2020.5655.

Shenhav A, Botvinick MM, Cohen JD. 2013. The expected value of control: an integrative theory of anterior cingulate cortex function. *Neuron*. 79:217–240. https://doi.org/10.1016/j.neuron.2013.07.007.

Shulman GL et al. 1997. Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. *J Cogn Neurosci*. 9:648–663. https://doi.org/10.1162/jocn.1997.9.5.648.

Spielberg JM et al. 2015. Pubertal testosterone influences threat-related amygdala–orbitofrontal cortex coupling. *Soc Cogn Affect Neurosci*. 10:408–415. https://doi.org/10.1093/scan/nsu062.

Sridharan D, Levitin DJ, Menon V. 2008. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc Natl Acad Sci*. 105:12569–12574. https://doi.org/10.1073/pnas.0800005105.

Stein M et al. 2021. Alcohol-related context modulates neural correlates of inhibitory control in alcohol dependent patients: preliminary data from an fMRI study using an alcohol-related go/NoGo-task. *Behav Brain Res*. 398:112973. https://doi.org/10.1016/j.bbr.2020.112973.

Swanson HL. 2004. Working memory and phonological processing as predictors of children's mathematical problem solving at different ages. *Mem Cogn*. 32:648–661. https://doi.org/10.3758/bf03195856.

Tamnes CK et al. 2017. Development of the cerebral cortex across adolescence: a multisample study of inter-related longitudinal changes in cortical volume, surface area, and thickness. *J Neurosci*. 37:3402–3412. https://doi.org/10.1523/JNEUROSCI.3302-16.2017.

Tyborowska A et al. 2024. Developmental shift in testosterone influence on prefrontal emotion control. *Dev Sci*. 27:e13415. https://doi.org/10.1111/desc.13415.

Vadakkadath Meethal S, Atwood CS. 2005. The role of hypothalamic-pituitary-gonadal hormones in the normal structure and functioning of the brain. *Cell Mol Life Sci*. 62:257–270. https://doi.org/10.1007/s00018-004-4381-3.

Vijayakumar N, Pfeifer JH, Flournoy JC, Hernandez LM, Dapretto M. 2019. Affective reactivity during adolescence: associations with

age, puberty and testosterone. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*. 117:336–350. https://doi.org/10.1016/j.cortex.2019.04.024.

White SF, Lee Y, Schlund MW, Shirtcliff EA, Ladouceur CD. 2020. Testosterone reactivity is associated with reduced neural response to reward in early adolescence. *Behav Brain Res*. 387:112593. https://doi.org/10.1016/j.bbr.2020.112593.

Yang Z et al. 2015. Intrinsic brain indices of verbal working memory capacity in children and adolescents. *Dev Cogn Neurosci*. 15:67–82. https://doi.org/10.1016/j.dcn.2015.07.007.

Ye F, Kohler R, Serio B, Lichenstein S, Yip SW. 2022. Task-based co-activation patterns reliably predict resting state canonical network engagement during development. *Dev Cogn Neurosci*. 58:101160. https://doi.org/10.1016/j.dcn.2022.101160.

Cerebral Cortex, 2025, **35**, bhaf218
https://doi.org/10.1093/cercor/bhaf218
Original Article