Journal of Developmental Origins of Health and Disease

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Developmental variation in testosterone: cortisol ratio alters cortical- and amygdala-based cognitive processes

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Original Article

Cite this article: Lew J, Jones SL, Caccese C, Orfi I, Little C, Botteron KN, McCracken JT, and Nguyen T-V. (2022) Developmental variation in testosterone:cortisol ratio alters cortical- and amygdala-based cognitive processes. *Journal of Developmental Origins of Health and Disease* **13**: 310–321. doi: 10.1017/S2040174421000362

Received: 17 April 2021
Revised: 2 June 2021
Accepted: 8 June 2021
First published online: 29 July 2021

Keywords:
Androgens; adolescence; cognition; TC ratio; brain development

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Abstract

Testosterone (T) and cortisol (C) are the end products of neuroendocrine axes that interact with the process of shaping brain structure and function. Relative levels of T:C (TC ratio) may alter prefrontal–amygdala functional connectivity in adulthood. What remains unclear is whether TC-related effects are rooted to childhood and adolescence. We used a healthy cohort of 4–22-year-olds to test for associations between TC ratios, brain structure (amygdala volume, cortical thickness (CTh), and their coordinated growth), as well as cognitive and behavioral development. We found greater TC ratios to be associated with the growth of specific brain structures: 1) parietal CTh; 2) covariance of the amygdala with CTh in visual and somatosensory areas. These brain parameters were in turn associated with lower verbal/executive function and higher spatial working memory. In sum, individual TC profiles may confer a particular brain phenotype and set of cognitive strengths and vulnerabilities, prior to adulthood.

Introduction

Testosterone and cortisol are steroid hormones and the end products of two intrinsically co-regulated brain–endocrine axes, the hypothalamic–pituitary–gonadal (HPG) and adrenal (HPA) axes. In certain contexts, testosterone inhibits cortisol release, while in others cortisol inhibits testosterone secretion.[1] As a result, relative levels of testosterone:cortisol, or TC ratios, are thought to represent a useful index of HPG–HPA interactions.[2–7]

Interestingly, research supports the existence of a link between TC ratio and alterations in brain structure and behavior. Testosterone in particular may be associated with lower cortical thickness (CTh), perhaps as a result of a decrease in neuronal growth and astrocyte recruitment in cortical gray matter.[8,9] On the other hand, it tends to promote white matter growth through oligodendrocyte-related myelination, enhancing the structural and functional connectivity within brain regions, in particular those implicated in language.[9–15].

Cortisol may be more neurotoxic than testosterone, particularly when chronically elevated levels are present. For example, severely abused and neglected children tend to exhibit both higher cortisol levels and lower cerebral and cerebellar gray matter volume (GMV), as well as a prominent GMV decrease in several key brain regions such as the prefrontal cortex.[16] While these data suggest that chronic elevation in cortisol levels related to stress can influence structural brain development in children, it remains unclear whether these developmental effects can be moderated or compensated by other hormones, such as testosterone.

These stress-related effects have been linked with the activation of the amygdala, as a central hub of the HPA axis circuitry with a high density of corticotropin-releasing factor (CRF) and glucocorticoid receptors.[17–20] Notably, androgen receptors (ARs) can be found in amygdaloid neurons just adjacent to the CRF-expressing neurons of the amygdala.[21–24] Even within the same developmental window, the impact of testosterone may depend on the specific brain region and cognitive domains of interest.[25–27] For example, testosterone was found to adversely affect executive function in male children and adolescents, an effect mediated by differences in prefrontal–hippocampal structure.[23] Yet in slightly older young adults, testosterone was linked to *better* visuospatial abilities.[28] In contrast to these mixed findings, higher cortisol levels have been consistently linked to poorer executive functioning,[29,30] impairments in working

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memory,[29] and a higher risk of internalizing and externalizing disorders[31–33] across a variety of different studies.

Taken together, the current literature suggests that variation in the relative levels of testosterone and cortisol may support individual differences in amygdala and cortical structures[2,34] as well as in the connections between these regions.[21,35–37] These differences in brain structure might in turn contribute to the cognitive and behavioral profile associated with those hormones in adulthood: 1) a testosterone-related impairment in executive function combined with greater visuospatial cognitive abilities[2] and 2) a cortisol-related increase in fearfulness or withdrawal behaviors.[38] As a result, greater TC ratios may be involved in the facilitation of both reactive and instrumental aggression in adults with a prior history of violence.[3,39,40]

Still, information is lacking as to how variations in TC ratio during earlier development might lead to such divergent outcomes in adulthood, that is, predisposing some individuals to impulsive and proactive aggression, while optimizing complex cortical functions in others with little/no attendant expression of violence. One possibility is that early developmental differences in TC ratio trigger discrepant growth trajectories in the amygdala and cortex. In turn, these structural brain differences may lead to a reinforcement of *either* beneficial or detrimental hormonal effects during adulthood.

Thus, this longitudinal study aimed to examine the relationships between TC ratios and brain growth (amygdala volume, whole-brain CTh, and coordinated growth, or covariance between the amygdala and the cortex), as well as cognitive and behavioral development from 4 to 22 years old, based on the following hypotheses: 1) greater TC ratios will be associated with greater amygdala volumes and CTh in regions with a high density of steroid receptors, such as the prefrontal, visuomotor and somatosensory regions; 2) greater TC ratios will facilitate greater covariance between the amygdala and prefrontal, visuomotor and somatosensory regions; and 3) structural brain differences associated with greater TC ratios will confer on a given child a predisposition for aggression, characterized by impaired executive function, higher visuospatial skills, and lower frequency of anxious-depressed symptoms.

Methods and materials

Sampling and recruitment

The data used in this study were obtained from the National Institute of Health magnetic resonance brain imaging (MRI) Study of Normal Brain Development, a multi-site project that aims to provide a normative database to characterize healthy brain maturation. Subjects ($n = 433$) were recruited across the USA with a population-based sampling method to achieve a demographically representative sample relative to income level, race, and ethnicity.[41] Subjects underwent repeated MRI every 2 years, with a maximum of three scans over 4 years. The sample was limited to developmentally healthy children and used rigorous exclusion criteria to select for developmentally healthy English-speaking children. Exclusion criteria included pre and perinatal factors known to disrupt brain development (e.g., maternal smoking, drinking or drug use during pregnancy, obstetric complications, and pre-term birth), physical/medical or growth characteristics (e.g., height or weight below the 3rd percentile), and history of neurological disorders or abnormal neurological exams. Behavioral/psychiatric assessments were used to exclude children with a current or past treatment for language disorder (simple articulation disorders not exclusionary), a lifetime history of Axis I psychiatric disorder (except for simple phobia, social phobia, adjustment disorder, oppositional defiant disorder, enuresis, encopresis, nicotine dependency) or any Child Behavior Checklist (CBCL) subscale score ≥70, Wechsler Abbreviated Scale of Intelligence <70, Woodcock–Johnson III Achievement Battery subtest score <70. Additional details are described elsewhere.[41] After strict quality control of MRI data (see the "Neuroimaging measures" section) and exclusion of scans with missing hormonal measurements or cognitive/behavioral parameters, a total of 225 subjects aged between 4.88 and 22.28 years old were used for hormone-related analyses (totalling 354 scans) (see Table 1). All experiments on human subjects were conducted in accordance with the Declaration of Helsinki. All procedures were carried out with participants' adequate understanding of the research protocol and after obtention of parental written consent, as well as verbal assent from subjects under 18 years old. If subjects were above the age of 18, their written consent was acquired.

Neuroimaging measures

A three-dimensional T1-weighted Spoiled Gradient Recalled echo sequence from 1.5 Tesla scanners was obtained on each participant, with 1 mm isotropic data acquired sagittally from the entire head for most scanners. In addition, T2-weighted and proton density-weighted images were acquired using a two-dimensional multi-slice (2 mm) dual echo fast spin echo sequence. Fully automated analysis of whole-brain CTh was performed through the CIVET pipeline, developed at the Montreal Neurological Institute. First, a multistage quality control process was implemented by excluding subjects with white or gray matter artifacts. All quality-controlled MR images were subsequently processed through the CIVET pipeline. These processing steps have been described at length in other publications.[8,21]

Volumetric measures of the amygdala were obtained from MRI data using a fully automated segmentation method validated in human subjects.[42] This method utilizes a large, manually labeled MRI dataset ($n = 80$) of young healthy adults that serves as a template library.[43] The manual segmentation was completed by four different raters, and intra-class, intra-rater, and inter-rater reliability varied between 0.83 for the right and 0.95 for the left amygdala.[44] From this manual segmentation, a fully automated method was derived, characterized by label fusion techniques that combine segmentations from a subset of "n" most similar templates. Specifically, each template is used to produce an independent segmentation of the subject using the ANIMAL pipeline,[45] followed by a thresholding step to eliminate cerebrospinal fluid (CSF), which results in "n" different segmentations. To fuse the segmentations at each voxel, a voting strategy is used; the label with the most votes from the "n" templates is assigned to the voxel. Combining multiple segmentations minimizes errors and maximizes consistency between segmentations. When using $n = 11$ templates, the label fusion technique has been shown to yield an optimal median Dice Kappa of 0.826 and Jaccard similarity of 0.703 for the amygdala.[42]

Hormonal and pubertal measures

During each research visit, children provided two 1–3 cm^3 samples of saliva before and after neurocognitive testing (totaling four samples), which were assayed by enzyme-linked immunosorbent assay (ELISA), and the average was used to compute hormonal

https://doi.org/10.1017/S2040174421000362 Published online by Cambridge University Press

Table 1. Sample characteristics at each visit included in the testosterone:cortisol (TC ratio) analyses

	Visit 1 ($n = 126$, 53M, 73F)	Visit 2 ($n = 128$, 48M, 80F)	Visit 3 ($n = 100$, 45M, 55F)
	M ± SD [freq] (range)	M ± SD [freq] (range)	M ± SD [freq] (range)
Age (years)	12.48 ± 3.29 (4.88–18.25)	13.27 ± 3.61 (6.80–20.19)	14.41 ± 3.68 (9.08–22.28)
Testosterone at time 1 (pg/dl)	66.90 ± 54.30	50.35 ± 31.32	69.53 ± 73.99
Testosterone at time 2 (pg/dl)	60.37 ± 46.50	45.03 ± 29.80	67.90 ± 61.42
Cortisol at time 1 (pg/dl)	22.16 ± 17.97	17.05 ± 11.34	20.81 ± 18.87
Cortisol at time 2 (pg/dl)	15.40.16 ± 11.61	11.16 ± 7.77	14.95 ± 12.53
Estradiol at time 1 (pg/dl)	7.49 ± 4.17	8.0 ± 4.33	10.96 ± 6.88
Estradiol at time 2 (pg/dl)	7.17 ± 4.42	8.0 ± 4.69	11.53 ± 6.91
DHEA at time 1 (pg/dl)	102.23 ± 101.81	195.14 ± 204.82	179.36 ± 160.26
DHEA at time 2 (pg/dl)	96.05 ± 111.34	173.64 ± 179.29	207.20 ± 210.30
Season of sampling	[38 spring; 49 summer; 16 fall; 23 winter]	[33 spring; 48 summer; 25 fall, 22 winter]	[33 spring; 38 summer; 13 fall, 16 winter]
Collection time 1 (mins after midnight)	680.09 ± 137.23	711.00 ± 123.49	710.36 ± 130.26
Collection time 2 (mins after midnight)	819.21 ± 152.99	867.77 ± 98.77	862.64 ± 106.61
Pubertal stage	2.13 ± 1.16	2.30 ± 1.28	2.64 ± 1.37
Handedness	[L = 11, R = 115]	[L = 11, R = 117]	[L = 9, R = 91]
Total brain volume–gray matter volume (cm^3)	486.93 ± 63.96	496.73 ± 65.06	512.56 ± 74.86
Left hippocampus (mm^3)	2940.10 ± 324.23	2985.47 ± 305.35	3087.75 ± 345.36
Right hippocampus (mm^3)	3028.87 ± 354.48	3072.68 ± 330.18	3153.42 ± 371.69

F, female; M, male; DHEA, dehydroepiandrosterone; L, left; R, right.
Total number of scans = 354 (208 females). 123 participants (68 females) were scanned only once, 75 (40 females) were scanned twice, and 27 (20 females) were scanned three times. Season of sampling was coded as spring, summer, fall, or winter.

levels. Care was taken to collect saliva samples within 1–2 hours after the completion of the MRI scan. Most measures were collected in the early morning and early afternoon, though no waking samples were collected *per se*. The intra-assay and inter-assay coefficients of variation were 6.5% and 16.2% for dehydroepiandrosterone (DHEA), 3.1% and 7.2% for cortisol, 6.1% and 13.5% for testosterone, and 4.1% and 9.1% for estradiol, respectively (Salimetrics Salivary ELISA Kit, State College, PA). At the following visit, a similar procedure was followed; two saliva samples were collected at each time, before and after the neurocognitive testing for hormonal measurement.

Salivary sampling measures the non-protein-bound, free, biologically active portions of circulating hormonal levels. These portions freely cross the blood–brain barrier and are therefore more relevant than blood plasma hormonal levels in studies of brain–hormone associations.[46,47] Salivary testosterone levels show moderate correlations with serum and CSF levels.[48,49] Levels of testosterone have also been shown to follow season and diurnal patterns in response to the pulsating release of adrenocorticotropic hormone and gonadotropin-releasing hormone. This variation in testosterone release is particularly apparent in males.[50,51] Similarly, salivary cortisol levels correlate highly with serum levels which, in turn, correlate highly with ventricular CSF levels. Cortisol shows marked reactivity to the environment, including diurnal rhythms, with morning levels up to 10-fold those collected in the evening.[51] To control for the environmental reactivity of testosterone and cortisol, time of sample collection, season, and sex have been included as covariates in hormonal-related analyses (see the "Statistical analyses" section).

TC ratio collected at time 1 (before the neurocognitive testing) was evaluated in separate models than TC ratio collected at time 2 (after the neurocognitive testing). This separation was performed as the two collection times represent different measures of stress response, that is, anticipatory stress increase versus post-stress reactivity. Indeed, cognitive testing in itself may constitute a stressful situation, eliciting a cortisol response. Elevations in salivary cortisol have previously been reported in response to cognitive testing specifically,[52] suggesting that hormonal measures collected before and after neurocognitive testing constitute different responses to stress. Testosterone and cortisol area-under-the-curve (AUC) with respect to ground were also calculated, as AUC may represent a better measure of total hormonal output, both in terms of magnitude and reactivity.[53] Testosterone AUC was divided by cortisol AUC to yield a TC AUC ratio.

To measure pubertal maturation, the Pubertal Development Scale (PDS) was clinically administered to all subjects during patient interviews.[54] The PDS has been shown to have good reliability (coefficient alpha: 0.77) and validity ($r^2 = 0.61$–0.67) compared to physical examination.[54] Because many of the physical changes seen during puberty are attributable to testosterone levels, which rise during gonadarche (e.g., facial hair and voice changes in boys), controlling for overall pubertal stage may diminish existing relationships between testosterone and the outcomes of interest. To better account for pubertal changes not related to gonadarche and testosterone levels, we created a puberty variable that measured physical changes related to adrenarche, based on questions related to body hair and skin changes. Residuals of TC ratio, controlled for adrenarche, were used in hormonal analyses.

https://doi.org/10.1017/S2040174421000362 Published online by Cambridge University Press

Cognitive measures

To measure internalizing and externalizing symptoms, we selected the Anxious-Depressed, Aggression and Rule-Breaking subscales from the CBCL and Young Adult Self-Report (YASR). The CBCL and YASR are age-appropriate instruments extensively used for assessing psychopathology and competence worldwide and ask parents or young adults themselves to report on specific behaviors exhibited within the last 6 months.[55,56] The YASR was derived from items on the CBCL and serves as a self-report extension of the CBCL for young adults.[57] Both the CBCL and YASR are reliable measures with high stability over time, validated in multiple cultures, and with high internal consistency.[56]

To measure executive function, we selected the Behavior Rating Inventory of Executive Function (BRIEF). The BRIEF uses parental ratings of executive function in the context of everyday problem-solving. It measures executive function including working memory in an integrated and relativistic way, outlining the complex, priority-based decision-making that is demanded in real-world situations.[58–61] The main strength of the BRIEF lies in its use of ecologically valid measurements, which allow a "real-world" snapshot of working memory that includes aspects of complex, everyday problem-solving demands.[58, 59] The BRIEF has demonstrated high test–retest reliability ($r \approx 0.70$–0.89 for parent ratings) and high internal consistency (Cronbach's alphas $\approx$ 0.80–0.98).[58]

To measure verbal learning and comprehension, we used the California Verbal Learning Test-Children's Version (CVLT-C)/ California Verbal Learning Test-II (CVLT-II) and the Woodcock–Johnson III Tests (WJ-III). CVLT is a laboratory-based cognitive test evaluating verbal learning and working memory.[62–65] This test measures performance with regard to semantic clustering, serial clustering, free versus cued recall, perseveration and intrusion errors, response bias, response consistency, and learning slope and yields several sub-scores, of which five of them are particularly relevant to verbal memory: long-delay (cued and free recall), short-delay (cued and free recall), and total number of words recognized. The CVLT is one of the most frequently used measures of verbal learning and memory in children and has high reliability: $r \approx 0.62$–0.93.[66] The previously demonstrated construct validity and temporal stability of the CVLT also make it a good measure of episodic verbal learning and memory as supported by a considerable body of research.[67,68] WJ-III is a rigorously developed and reliable assessment, with test–retest reliability ranging from 0.80 to 0.90 for the subtests we administered: Letter-Word Identification and Passage Comprehension, representing distinct measures of verbal and language skills.[69]

Finally, to measure spatial working memory, we used the Cambridge Neuropsychological Test Automated Battery (CANTAB), namely the CANTAB Spatial Working Memory subtest, including the use of self-guided spatial search strategies and error rate, that is, number of misses-omission errors and false alarms-commission errors. The CANTAB is a computerized battery that includes only nonverbal geometric designs or simple shapes, with minimal required language proficiency.[69,70] The validity of the CANTAB for assessing brain–behavior relations in adults has been established, and results of tests in pediatric populations showed that children can be tested with the same item sets that are employed in adult studies.[69,70] Reliability is high in pediatric populations (internal consistency coefficients = 0.73 for reaction time and 0.95 for performance on the spatial working memory test) and construct validity has been established in pediatric populations.[69,70]

Statistical analyses

Statistical analyses were performed using SurfStat (Matlab toolbox designed by Keith J. Worsley; http://www.math.mcgill.ca/keith/surfstat/) and SPSS 25.0 (SPSS, Inc., Chicago, Illinois). Additional covariates were introduced to the models to control for factors that may affect hormonal levels. These covariates included age, sex, total brain volume (TBV), and handedness. Handedness was coded as left, right, or mixed using Unimanual Laterality Index[71] and was assessed using 12-item performance (for 4:6–5:11 aged group) or 8-item pantomime performance scales (for 6 years and older group) (see https://www.bic.mni.mcgill.ca/nihpd_info/ for more details). TBV was defined as: (total GMV + total white matter volume + CSF inside the brain) − (CSF found outside the brain (but within the skull)). All continuous variables were centered using their respective means.

TC ratio and amygdala volume

Mixed-effects statistical analysis models were used to examine the association between TC ratio and amygdala volume. Separate models were performed to examine the association between TC ratios computed with left, right, and mean amygdala volume. A model was considered significant if it was below the threshold for significance ($P = 0.05$).

TC ratio and CTh

To model the relationship between TC ratio and whole-brain CTh, mixed-effects statistical designs were used. A correction for multiple comparisons across the whole brain was performed on all analyses using random field theory (RFT) ($P < 0.05$).[72] To examine any distinct effects of testosterone and cortisol above and beyond those related to estradiol, DHEA, or season of collection, these variables were also included as control variables in additional models. Pubertal stage (representing adrenarche) was accounted for using residuals of TC ratio controlled for adrenal stage.

TC ratio and cortico-amygdala structural covariance

Mixed-effects statistical analyses were used to model the relationship between T1, T2, and TC AUC ratio, and covariance of amygdala volume with whole-brain CTh. A correction for multiple comparisons across the whole brain using RFT ($P < 0.05$) was applied to all analyses.[72] The significance of the term "TC ratio×Amygdala" was examined while controlling for the previously mentioned control variables. If initial significance was determined, the DHEA and estradiol levels relevant to each timepoint as well as the season of collection were added as additional control variables in separate models to examine the effects of testosterone and cortisol above and beyond those related to these variables. Pubertal stage (representing adrenarche) was accounted for using residuals of TC ratio controlled for adrenal stage.

TC-related differences in brain structure and cognition/behavior

We tested for the associations between cognitive/behavioral measures and brain regions found to be significantly associated with TC ratios and CTh (refer back to the "TC ratio and CTh" section), as well as the significance of the term "CTh×Amygdala", for areas

https://doi.org/10.1017/S2040174421000362 Published online by Cambridge University Press

found to be significant in the TC-related structural covariance analyses (refer back to the "TC ratio and cortico-amygdala structural covariance" section). All analyses were controlled for age, sex, TBV, scanner, and handedness.

Indirect effects of TC ratio on cognition/behavior

To formally test whether TC ratio may indirectly affect cognitive/behavioral development through a variation in brain structure, we extracted the beta coefficients and standard errors from: (1) the associations between TC ratio-brain structure (from analyses described in the "TC ratio and amygdala volume," "TC ratio and CTh," and "TC ratio and cortico-amygdala structural covariance" sections); (2) the associations between brain structure and cognitive/behavior measures (see the "TC-related differences in brain structure and cognition/behavior" section); and (3) the associations between TC ratio and cognitive/behavioral measures found to be significantly associated with brain parameters in the "TC-related differences in brain structure and cognition/behavior" section. We then entered these beta coefficients and standard errors in a calculator using different versions of the Sobel–Goodman test to test for mediation effects of brain covariance (http://quantpsy.org/sobel/sobel.htm). This traditional approach to test indirect effects – using Baron–Kenney's criteria and augmented by a formal Sobel's test – was preferred to more recent methods that include bootstrapping. This is because of the complexity of our longitudinal data (multiple scans per subject, different number of scans per subject). The traditional method treats each relationship (between predictor and mediator, and then between mediator and outcome) separately, allowing us to model the longitudinal component of the data. Finally, the same set of control variables as listed in the "TC ratio and cortico-amygdala structural covariance" and "TC-related differences in brain structure and cognition/behavior" sections (i.e., age, sex, TBV, scanner, and handedness) were used for this section of analyses.

Results

Sample characteristics

Table 1 provides the details of sample characteristics, including the number of longitudinal scans and covariates of interest. The samples used for analyses included 225 participants, with 128 female (F) subjects. There was a total of 354 scans ($F = 208$). Participants were between 4.88 and 22.28 years old, with a mean age of 12.48 years (SD = 3.29) on visit 1, 13.27 (SD = 3.61) on visit 2, and 14.41 (SD = 3.68) on visit 3. Each subject was followed longitudinally up to three times, every 2 years, for a total of 4 years.

TC ratio and amygdala volume

No association was found between TC ratio and amygdala volume (Ps > 0.05).

TC ratio and CTh

TC AUC ratio was positively associated with CTh of the right superior parietal lobule (Brodmann Area 7; BA 7) (Fig. 1, panels a and b; Table 2). This association remained significant when controlling for adrenal pubertal status as measured by PDS. Adding estradiol, DHEA, and season of sampling as control variables also did not alter these findings. Neither TC ratio collected at time 1 or 2 was associated with CTh. No other associations were found between hormones and whole-brain native space CTh.

TC ratio and cortico-amygdala structural covariance

TC AUC ratio was positively associated with structural covariance between the mean amygdala volume and CTh of the right somatosensory cortex (BA 1/2/3) (Fig. 2, panels a and b; Table 3), and right primary as well as visual association areas (BA 17/18) (Fig. 3, panels a and b). Adding season of sampling, estradiol, and DHEA as control variables did not change the significance of the findings. Neither TC ratio collected at time 1 or 2 was associated with structural covariance between amygdala volume and CTh. No other associations were found between hormones and structural covariance of amygdala volume and other CTh regions.

TC-related brain structure and cognition/behavior

CTh in the area of the superior parietal lobule (which varied as a function of TC AUC ratio as shown in the "TC ratio and CTh" section) was negatively associated with tests of verbal recall and verbal comprehension. Specifically, higher CTh in the superior parietal lobule was associated with lower scores on two CVLT tests of number recall (long-delay cued and short-delay free verbal recalls) (Fig. 1, panels c and d, respectively) as well as lower scores on the WJ-III passage comprehension test (Fig. 1, panel e). There was no significant association between TC AUC-related CTh in the superior parietal lobule and behavioral measures.

Covariance between visuo-amygdala and somatosensory–amygdala brain regions (which varied as a function of TC AUC ratio; see the "TC ratio and cortico-amygdala structural covariance" section) predicted scores on tests of spatial working memory (CANTAB) and executive function (BRIEF organization). Specifically, visuo-amygdala structural covariance was associated with higher spatial working memory, that is, lower error rate on the CANTAB spatial working memory subtest (Fig. 3, panel c). Somatosensory–amygdala structural covariance was also associated with higher spatial working memory (Fig. 2, panel c). In addition, somatosensory–amygdala structural covariance predicted lower executive function, as measured by BRIEF organization scores (Fig. 2, panel d). There was no significant association between TC AUC-related cortico-amygdala structural covariance and behavioral measures.

Indirect effects of TC ratio on cognition and behavior

There were no significant indirect effects of TC AUC ratio on verbal working memory and verbal comprehension through CTh of the superior parietal lobule (see Table 4). There were significant indirect effects of TC AUC ratio on spatial working memory (as measured by CANTAB error scores) through somatosensory–amygdala covariance ($P = 0.050$, see Table 5), but only trends for indirect effects of TC AUC ratio on executive function (as measured by BRIEF organization scores) through somatosensory–amygdala covariance ($P = 0.057$) and for indirect effects of TC ratio on spatial working memory through visuo-somatosensory covariance (CANTAB; $P = 0.068$).

Discussion

Taken together, our findings provide further support about the effects of relative levels of testosterone and cortisol on the development of the cortex and the amygdala. We find that TC AUC levels influence CTh of the superior parietal lobule as well as the coordinated growth of visual/somatosensory cortical areas and amygdala volume. In turn, differences in these brain structures

https://doi.org/10.1017/S2040174421000362 Published online by Cambridge University Press

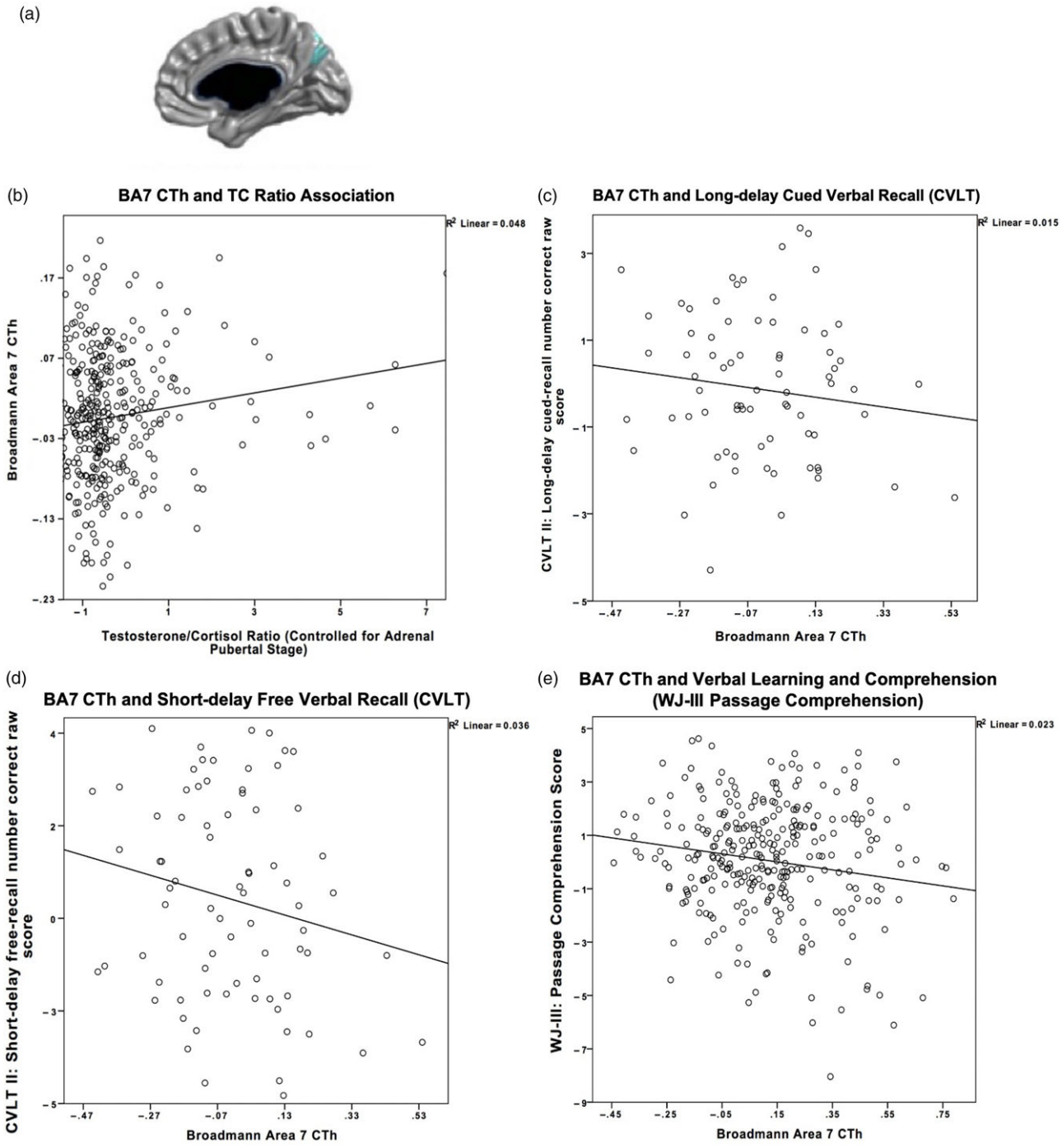


Fig. 1. Testosterone:cortisol ratio relationship with superior parietal lobule cortical thickness and verbal learning/comprehension. This figure shows the association between testosterone:cortisol (TC) ratio levels, whole-brain cortical thickness (CTh) (Broadmann Area 7/BA7 shown in panel (a) and their association shown in panel (b)), long-delay cued verbal recall (panel (c)), short-delay free verbal recall (panel (d)), and verbal comprehension (panel (e)). Panel (a) shows the region of the right superior parietal lobule (BA7) where a positive association between TC ratio and native space, whole brain CTh was found when controlling for adrenarche (surviving corrections for multiple comparisons across the whole brain using random field theory, $P < 0.05$). Panel (b) displays the positive association between TC ratio (controlled for adrenal pubertal stage) and whole brain, native space CTh of the superior parietal lobule (BA7). Figure 1 also displays the negative relationships between TC-related CTh of the superior parietal lobule (BA7) and verbal learning as well as comprehension scores on the CVLT II: long-delay cued verbal recall test (panel (c)), CVLT II: short-delay free verbal recall test (panel (d)), and WJ-III: passage comprehension test (panel (e)).

contribute to a specific cognitive profile characterized by lower executive function and verbal learning/comprehension and greater spatial working memory.

Our hypotheses were only partially confirmed. For example, a higher TC AUC ratio was indeed associated with greater CTh in the superior parietal lobule but not with amygdala volume. Higher TC AUC ratios were also associated with greater coordinated growth between visuo- and somatosensory cortical regions and amygdala volume (i.e., positive structural covariance) but did not influence prefrontal–amygdala covariance as originally

https://doi.org/10.1017/S2040174421000362 Published online by Cambridge University Press

Table 2. TC ratio, CTh of the superior parietal lobule (BA7), and cognition

	Beta coefficient	Standard error	*P*-value
TC ratio and BA7 CTh	0.105	0.054	Peak vertex: 2.02×10^{-6}
			Cluster-level $P < 0.05$
BA7 CTh and WJ-III passage comprehension	−3.280	1.364	0.017
BA7 CTh and CVLT long-delay cued-recall	−3.289	1.446	0.028
BA7 CTh and CVLT short-delay free-recall	−4.040	1.878	0.037

TC ratio, testosterone AUC:cortisol AUC ratio; WJ-III, Woodcock–Johnson Test; CVLT, California Verbal Learning Test.

predicted. Finally, the influence of TC AUC ratios on developmental brain trajectories may contribute to a certain cognitive predisposition for externalizing symptoms and aggressive behaviors, characterized by a combination of impaired verbal and executive functions as well as high visuospatial skills. However, there were in fact no direct associations between TC ratios and behavioral measures (including internalizing or externalizing symptoms) and only weak indirect relationships between TC ratios and cognition.

Thus, TC AUC-related structural changes seem to preferentially impact specific brain regions, and not others. In turn, differences in brain structure predominantly affect distinct cognitive domains with little effect on behavior. The higher sensitivity of these brain regions to variation in TC AUC ratios may reflect a greater overall neuroplasticity in these areas to endogenous hormonal levels during childhood and adolescence, as well as their importance in the development of core verbal, spatial, and executive abilities.

This is evident in the association between greater TC AUC ratios and higher CTh of the superior parietal lobule (BA7). Much of the output of this cortical area projects to the dorsolateral prefrontal cortex, the frontal motor cortex, and the frontal eye fields[73] to integrate auditory-verbal and visuospatial information.[74–76] In our study, CTh within this area was associated with tests of verbal learning and comprehension, consistent with the importance of the superior parietal cortex in the manipulation of auditory-verbal information in working memory.[75] However, CTh of the superior parietal lobule was not associated with tests of spatial working memory, perhaps due to other effects of the TC AUC ratio on cortico-amygdala structural covariance.

Indeed, TC AUC-related visuo-amygdala and somatosensory–amygdala covariances were associated with better retention and manipulation of visuospatial information, as measured by a lower number of omission and commission errors in a test of spatial working memory. This test has significant executive function demands and provides a measure of spatial strategy as well as working memory errors. The influence of TC AUC ratio on visual and somatosensory cortical areas may be explained by their high density of ARs,[77,78] which may also enhance coordination in their neural growth and that of the AR-rich amygdala.[79] In addition, recent evidence supports the notion that amygdala activation may increase plasticity in sensory regions such as the visual and somatosensory cortices.[80] This bottom-up process (amygdala to visual/somatosensory areas) may in turn help in optimizing spatial working memory by facilitating the discrimination between salient and irrelevant stimuli.[81]

Somatosensory–amygdala structural covariance was also associated with BRIEF organization scores. This parent-rated scale evaluates a child's orderliness in the context of work and play.[58] In this case, TC AUC-related covariance was associated with lower executive function (i.e., organizational abilities), similar to previous findings from our group showing a detrimental effect of testosterone levels on different aspects of executive function (i.e., monitoring and shifting abilities) in boys.[23] Of note, these previously reported relationships were mediated by differences in *prefrontal–hippocampal* structural covariance. Thus, TC-related influences on somatosensory–amygdala structural covariance may augment those of testosterone on prefrontal–hippocampal covariance, perhaps putting boys particularly at risk for executive impairment across several domains.

From an endocrine perspective, it is interesting to note that it is the ratio between total hormonal outputs that drove our findings, while no effects were detected when considering pre- or post-hormonal ratios in isolation. In other words, only TC AUC ratio, calculated using the hormonal values of all the samples collected before and after neurocognitive testing, was significantly associated with alterations in brain structure and cognition. This supports the notion that it is the hormonal trajectory and hormonal reactivity of an individual – even if only sampled over a period of a couple of hours – that may best predict that child's developmental potential as opposed to cross-sectional hormonal measures.

Finally, although lateralization effects can be challenging to interpret, it may be worth noting that all the cortical regions associated with TC ratios were in the right hemisphere, and that findings were slightly more significant with the right (compared to the left) amygdala. One could speculate that the right hemisphere may be more sensitive to the combined influence of testosterone and cortisol during this period of life (compared to the left). Although there is some evidence supporting this hypothesis,[82,83] our study cannot definitively answer any questions regarding hormonal-based lateralization effects.

Strengths and limitations

Our study includes a large, population-based sample, following a longitudinal design with repeated collection of hormonal, neuroimaging, cognitive, and behavioral measures. These strengths support the generalizability of our findings across a wide developmental window from childhood to early adulthood. Conversely, we find direct and indirect effects of TC AUC ratio on cognition to be minimal or to not reach significance, with the only significant indirect effect involving TC AUC ratio, somatosensory–amygdala structural covariance, and spatial working memory. Further, TC-related differences in brain structure were not associated with internalizing or externalizing symptoms (level and frequency of anxious-depressed symptoms, aggression, or rule-breaking behaviors). Taken together, these negative findings suggest that, in fact: 1) TC-related effects on aggression and other externalizing disorders previously reported in adult populations may be predominantly activational (i.e., mediated by reversible, transient changes in brain function) rather than organizational (mediated by relatively long-lasting or even irreversible changes in brain structure) and

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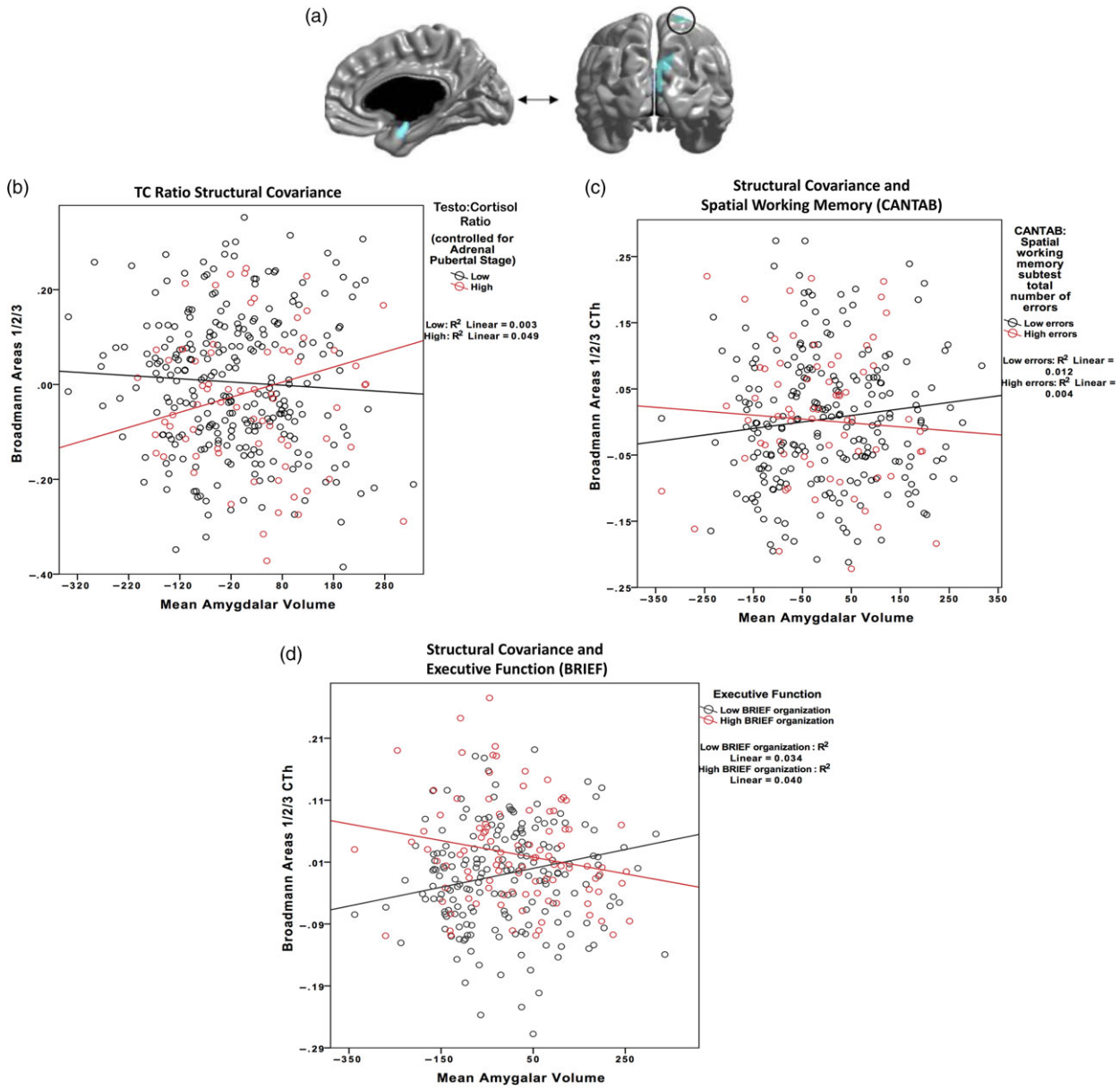


Fig. 2. Testosterone:cortisol ratio-related somatosensory–amygdala structural covariance and relationship with spatial working memory and executive function. This figure shows somatosensory–amygdala structural covariance related to testosterone:cortisol (TC) ratio levels and to scores on spatial working memory and executive function tests. Panel (a) shows the region of the right somatosensory cortex (Brodmann 1/2/3) where the relationship between cortical thickness (CTh) and amygdala volume (average of the left and right amygdala volumes) significantly varies according to TC ratio levels (surviving corrections for multiple comparisons across the whole brain using random field theory, $P < 0.05$). Panel (b) shows the relationship between amygdala volume (X axis) and CTh of the somatosensory cortex (Y axis) for subjects with lower TC ratio (black line) and higher TC ratio (red line) levels. Higher TC ratios were associated with a more positive somatosensory–amygdala structural covariance. Panel (c) displays the relationship between amygdala volume and CTh of the somatosensory cortex for subjects with *better* spatial working memory scores, that is, *low* error rates on the CANTAB spatial working memory test (black line), and *lower* spatial working memory scores, that is, *high* error rates on the CANTAB spatial working memory test (red line). Panel (d) displays the relationship between amygdala volume and CTh of the somatosensory cortex for subjects with *lower* executive function as measured by BRIEF organization (black line) and *higher* executive function (red line). Positive somatosensory–amygdala structural covariance (seen with greater TC ratios) was associated with *better* spatial working memory (panel (c)) and *lower* executive function (panel (d)). Note that the data are split into lower/higher TC ratios (panel (b)), lower/higher CANTAB error (panel (c)) and lower/higher BRIEF (panel (d)) scores only for illustrative purposes.

2) TC ratio can at most confer certain cognitive strengths and vulnerabilities, with the environmental, social, psychological, genetic context ultimately determining the final behavioral outcomes in an individual child. Interestingly, our effects were only detected with the TC AUC ratios and not the individual hormone measures (i.e., pre- and post-neurocognitive testing). This suggests that it is the total hormone output, or stress reactivity, that is important in the development of certain cortical regions and in amygdala–cortical structural covariance measures. Developmental studies should ensure to capture repeated hormone samplings and consider the total hormone output to better understand the role of hormones on neurocognitive development. Given the large set of analyses, independent replication of main effects is recommended to ensure validity.

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Table 3. TC ratio, cortico-amygdala covariance, and cognition

	Beta coefficient	Standard error	*P*-value
TC ratio and covariance:			Peak vertices:
1) Visuo-amygdala	0.081	0.054	2.11×10^{-3}
2) Somatosensory–amygdala	0.203	0.054	4.12×10^{-5}
			Cluster-level $P < 0.05$
Visuo-amygdala covariance and CANTAB errors	−0.008	0.004	0.046
Somatosensory–amygdala covariance and CANTAB errors	−0.007	0.003	0.028
Somatosensory–amygdala covariance and BRIEF organization	−0.028	0.013	0.035

TC ratio, testosterone AUC:cortisol AUC ratio; CANTAB, Cambridge Neuropsychological Test Automated Battery; BRIEF, Behavior Rating Inventory of Executive Function.

Conclusion

In sum, during childhood and adolescence, higher TC ratios may optimize sensory processing of spatial information through amygdala-dependent pathways to the cortex, to the detriment of overall executive and verbal functions requiring heavier cortical processing. Although these effects may prove to be adaptive later on during adulthood beyond the age range covered by our sample, our findings suggest that TC AUC ratio may represent a measure of risk and vulnerability to executive dysfunction during the pubertal transition.

Contribution statement

Jimin Lew and Sherri Lee Jones provided intellectual contributions to the rationale and interpretation of the present data, contributed to its presentation, edited the tables and figures, and prepared the manuscript for submission. Christina Caccese verified the presentation of data and prepared manuscript for submission. Isobel Orfi and Charlotte Little performed the data analyses and created the tables and figures. Kelly N Botteron and James T McCracken were responsible for the initial study design, data collection, and data processing. Tuong-Vi Nguyen conceived of the project, oversaw the data analyses, interpreted the data, provided intellectual contributions to the paper, and revised manuscript drafts. All authors approved the submitted manuscript.

Data Availability. The data supporting the findings of this study are available on request from the corresponding author. They are not publicly available due to privacy and ethical restrictions.

Acknowledgements. None.

Financial support. Jimin Lew received funding from the Research Institute of the McGill University Health Centre (RI-MUHC) studentship competition and the Fonds de Recherche du Québec – Santé (FRQS) Master's Training Award. Dr. Sherri Lee Jones received funding from Ferring Fellowship in Reproductive Health. Christina Caccese received funding from McGill University, Department of Psychiatry. Dr. Tuong-Vi Nguyen received funding

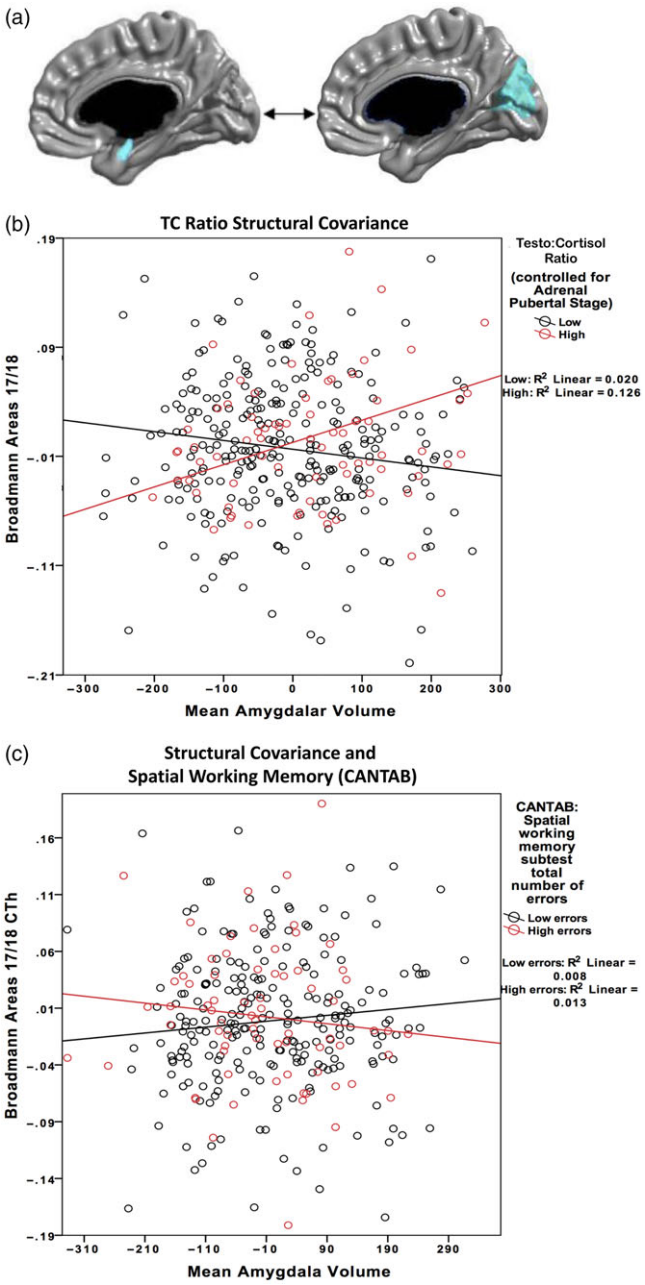


Fig. 3. Testosterone:cortisol ratio-related visuo-amygdala structural covariance and relationship with spatial working memory. This figure shows visuo-amygdala structural covariance related to testosterone:cortisol (TC) ratio levels and scores on spatial working memory tests. Panel (a) shows the region of the right occipital cortex (primary visual and visual association areas, Brodmann 17/18) where the relationship between cortical thickness (CTh) and amygdala volume (average of the left and right amygdala volumes) significantly varies according to TC ratio levels (surviving corrections for multiple comparisons across the whole brain using random field theory, $P < 0.05$). Panel (b) displays the relationship between amygdala volume (X axis) and CTh of the visual cortex (Y axis) for subjects with lower (black line) and higher TC ratio (red line) levels. Higher TC ratios were associated with a more positive visuo-amygdala structural covariance. Panel (c) displays the relationship between amygdala volume and CTh of the visual cortex for subjects with *better* spatial working memory scores, that is, *low* error rates on the CANTAB spatial working memory test (black line), and *lower* spatial working memory scores, that is, *high* error rates (red line). Positive visuo-amygdala structural covariance (as seen with higher TC ratios) was associated with *better* spatial working memory. Note that the data are split into lower/higher TC ratios and lower/higher CANTAB error scores only for illustrative purposes.

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Table 4. Formal tests of indirect effects of TC ratio on cognition through BA7 CTh

	Sobel–Goodman test statistic	*P*-value
TC ratio, BA7 CTh, WJ-III passage recall	1.595	0.111
TC ratio, BA7 CTh, CVLT long-delay cued-recall	1.567	0.117
TC ratio, BA7 CTh, CVLT short-delay free-recall	1.535	0.125

TC ratio, testosterone AUC:cortisol AUC ratio; WJ-III, Woodcock–Johnson Test; CVLT, California Verbal Learning Test.

Table 5. Formal tests of indirect effects of TC ratio on cognition through structural covariance

	Sobel–Goodman test statistic	*P*-value
TC ratio, somatosensory–amygdala covariance, CANTAB errors	1.955	0.050
TC ratio, somatosensory–amygdala covariance, BRIEF organization	1.901	0.057
TC ratio, visuo-amygdala covariance, CANTAB errors	1.823	0.068

TC ratio, testosterone AUC:cortisol AUC ratio; CANTAB, Cambridge Neuropsychological Test Automated Battery; BRIEF, Behavior Rating Inventory of Executive Function.

from the Montreal General Hospital and MUHC Foundations, FRQS, Canadian Institutes of Health Research, and the Merck-RI MUHC Biomedical Grants competition. Data collection for this study was also supported by Federal funds from the National Institute of Child Health and Human Development, the National Institute on Drug Abuse, the National Institute of Mental Health, and the National Institute of Neurological Disorders and Stroke (Contract #s N01-HD02-3343, N01-MH9-0002, and N01-NS-9-2314, -2315, -2316, -2317, -2319, and -2320).

Conflicts of interest. The authors declare no conflict of interest.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and have been approved by the institutional committees at the relevant clinical sites where recruitment took place: Boston Children's Hospital, Cincinnati Children's Hospital Medical Center, University of Texas Houston Medical School, Neuropsychiatric Institute and Hospital, University of California, Los Angeles, Children's Hospital of Philadelphia, and Washington University.

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https://doi.org/10.1017/S2040174421000362 Published online by Cambridge University Press