Sex Differences in Testosterone-Related Patterns of Cortical Maturation across Childhood and Adolescence

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ABSTRACT

Background:

Neuroendocrine theories of hemispheric lateralization hold testosterone as the predominant factor mediating sex-specific patterns of cortical growth and the ensuing lateralization of cognitive functions. However, to date attention has been mostly focused on prenatal testosterone levels as opposed to peripubertal changes in testosterone. Yet puberty coincides with both a significant rise in testosterone levels and increased differences between sexes in specific cognitive skills. Currently, direct examinations of relationships between testosterone and cortical grey matter remain limited, particularly in child or adolescent subjects. Weaknesses of the literature include small sample sizes, cross-sectional designs, uncorrected multiple comparisons, and the examination of restricted age spans. As a result, findings have been conflicting and vary across studies. Thus, the exact nature of testosterone-related sex-specific trajectories in cortical growth, timing of these effects throughout adolescence and brain regions most sensitive to testosterone, remain unclear.

Objective:

Examine testosterone-related sex differences in cortical thickness in a large, representative, longitudinal sample of healthy children/adolescents.

Methods:

Data from the NIH MRI Study of Normal Brain Development (n=282 subjects, 4-22 yo, 469 magnetic resonance imaging (MRI) scans obtained longitudinally over 4 years) were

analyzed using linear mixed effects models to examine the association between salivary testosterone levels and cortical thickness while controlling for age, sex, total brain volume, and collection time.

Results: We found significant and complex 'age by testosterone' and 'sex by testosterone' interactions on cortical thickness of widespread regions of both hemispheres, with striking differences between males and females as well as between younger pre-pubertal and older post-pubertal subjects. When males and females were tested separately, more pronounced regional effects emerged. Males showed a negative association between cortical thickness and testosterone levels exclusively in the left hemisphere, specifically in the posterior cingulate cortex starting at age 14, progressing as children age to include the precuneus (ages 16-19), dorsolateral prefrontal cortex (ages 16-21) and anterior cingulate cortex (ages 20-22). Females showed fewer areas of testosterone effects, but the associations exclusively involved the right hemisphere, specifically a positive association between cortical thickness and testosterone levels in the right somatosensory cortex, which was only apparent in the 5-8 years old age epoch, and later reversed, to a negative association in the same area for subjects ages 20-22.

Conclusions:

Significant sex- and age-specific associations exist between testosterone and cortical thickness in the developing brain but appear more complex than previously reported. While sex-specific testosterone-related cortical maturation appears to affect widespread areas in both hemispheres, more pronounced regional effects were observed in this study

in key cortical regions known to be implicated in visuospatial and sensorimotor skills as well as cognitive control and executive functions. Females also show early positive associations between testosterone and cortical thickness consistent with known protective effects of androgens on neurons. In addition, the male-specific association between testosterone and cortical thickness in the left hemisphere and the female-specific association with the right hemisphere support the view that sex differences in hemispheric lateralization progress in a dynamic fashion throughout puberty. Whether the observed testosterone-cortical thickness associations underpin age- and sex-related differentiation in behavior, psychopathology, or specific cognitive abilities during childhood and adolescence requires additional study.

RÉSUMÉ

Contexte:

Les théories neuroendocriniennes de latéralisation hémisphérique identifient la testostérone comme étant le facteur prédominant à l'origine des différences de développement cortical entre les sexes, ainsi que de la subséquente latéralisation des fonctions cognitives. Cependant, pour l'instant, une plus grande attention a été accordée à l'exposition intra-utérine à la testostérone plutôt qu'aux changements de testostérone autour de la puberté. Pourtant, la puberté coïncide avec une augmentation significative de la testostérone ainsi qu'une différence accrue entre les sexes en termes d'habiletés cognitives spécifiques. Présentement, les investigations directes des relations entre la testostérone et le cortex demeurent limitées, particulièrement en ce qui a trait aux enfants et aux adolescents. Les faiblesses de la littérature actuelle incluent de petits échantillons, des études transversales, plusieurs analyses sans correction pour comparaisons multiples, et l'inclusion de groupes d'âges limités. Par conséquent, les résultats demeurent conflictuels et varient selon les études. Ainsi, la nature exacte des trajectoires de développement cortical relié à la testostérone et le déroulement pendant l'adolescence de ces effets spécifiques à chaque sexe, ainsi que l'identification des régions du cerveau les plus sensibles à la testostérone, demeurent incertaines.

Objectif:

Examiner les différences de développement cortical entre les sexes reliés à la testostérone dans un large échantillon longitudinal d'enfants et d'adolescents représentatifs de la population générale.

Méthodes:

Des données du *NIH MRI Study of Normal Brain Development* (n=282 sujets, 4-22 ans, 469 résonances magnétiques obtenues longitudinalement sur 4 ans) ont été analysées en utilisant des modèles linéaires mixtes pour examiner la relation entre les niveaux de testostérone salivaire et l'épaisseur corticale tout en contrôlant pour l'âge, le sexe, le volume cérébral total et le temps de collection de la testostérone.

Résultats:

Nous avons trouvé des interactions significatives et complexes entre l'âge et la testostérone ainsi que le sexe et la testostérone sur l'épaisseur corticale de régions étendues des deux hémisphères, avec des différences frappantes entre les hommes et les femmes ainsi que les jeunes pré-pubères et les sujets post-pubères. Quand les hommes et les femmes ont été examinés séparément, des effets localisés plus prononcés ont émergé. Chez les hommes, il y avait une association négative entre la testostérone et l'épaisseur corticale exclusivement dans l'hémisphère gauche, plus spécifiquement dans le cortex cingulaire postérieur commençant à l'âge de 14 ans, progressant avec l'âge jusqu'à inclure le précuneus (âges 16-19 ans), le cortex dorsolatéral préfrontal (âges 16-21) et le cortex cingulaire antérieur (âges 20-22). Chez les femmes, il y avait moins de régions d'association avec la testostérone, mais ces associations affectaient exclusivement l'hémisphère droit, spécifiquement une association positive entre l'épaisseur corticale et la testostérone dans l'aire somatosensitive, seulement apparente de l'âge de 5 ans à 8 ans,

et s'inversant plus tard, devenant une association négative dans la même région pour les sujets d'âge 20 à 22 ans.

Conclusions:

Il y a des interactions significatives entre l'âge et la testostérone et le sexe et la testostérone sur l'épaisseur corticale qui semblent plus complexes que rapportées précédemment. Bien que le développement cortical spécifique à chaque sexe semble affecter des régions étendues dans les deux hémisphères, des effets localisés plus prononcés ont été observés dans cette étude dans des régions corticales connues pour être associées au contrôle cognitif et aux habiletés visuo-spatiales et sensorimotrices. Les femmes ont aussi démontré des associations positives avec le cortex somatosensitif qui sont consistantes avec les effets neuroprotecteurs des androgènes précédemment rapportés dans la littérature. De plus, la relation spécifique entre la testostérone et l'épaisseur corticale de l'hémisphère gauche chez les hommes et l'hémisphère droit chez les femmes supportent la représentation des différences entre les sexes en termes de latéralisation hémisphérique comme un processus dynamique qui progresse à travers la puberté. D'autres études plus poussées seront nécessaires pour confirmer si les associations observées entre la testostérone et l'épaisseur corticale sont véritablement reliés à une différentiation entre les sexes en termes de comportements, de psychopathologie et d'habiletés cognitives spécifiques au cours de la puberté.

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Disclaimer

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INTRODUCTION

Given the profound transformation that occurs during adolescence in physical, motivational, psychological, cognitive, and gender characteristics, increased interest in understanding the underlying relationships with brain development have emerged with the advent of non-invasive means of studying brain structure and function. Brain development across childhood and adolescence has been shown to be dynamic, and attempts have been made to identify specific linkages between discrete age-related changes and possible sex-related cognitive and behavior differences which may become more pronounced during adolescence[1-14].

The extent that sex differences in brain structure and function well described in adults are apparent early in development (as embryonic organizational effects), or emerge over time as later, additional organizing effects during puberty, remain not fully clear[1-14], although initial studies have suggested emerging sexual dimorphisms during adolescence, particularly in relationship to gray matter development[7-11]. These evolving sexual dimorphisms during adolescence are thought to be driven by hormonal changes throughout puberty[7-11].

Among the many possible hormonal influences on brain development occurring during puberty, changes in testosterone have been postulated as playing an important role in sex-specific differentiation of the brain. Indeed, the effect of testosterone is believed to

become significant as early as during the prenatal period, when masculinization of the fetal brain is thought to occur predominantly through testosterone crossing the blood-brain barrier and being subsequently aromatized to estradiol[15]. Estradiol, on the other hand, remains mostly bound in the periphery to alpha-fetoprotein and does not tend to cross the blood-brain barrier to the same degree as testosterone.

Because of the presumed role of hormones, particularly testosterone, in driving sex-related brain development, recent efforts have attempted to directly examine the relationships between testosterone and measures of brain structure and function[7-11]. However, the extant literature remains sparse and inconsistent and only limited conclusions can be drawn about testosterone-related anatomical grey matter development. Indeed, most reports examining testosterone-related effects on the human brain have used functional magnetic resonance imaging (fMRI) techniques or cross-sectional designs, and have been conducted with adult subjects, small sample sizes and restricted age spans[1-14].

Despite limitations of the current literature, testosterone remains the prime candidate in neuroendocrine theories of cortical development. One of the main tenets of Geschwind's theory of lateralization, elaborated more than 20 years ago, holds that testosterone is the most likely factor impacting sex-specific differences in cortical growth and the ensuing lateralization of cognitive functions to the left or the right hemisphere[16]. Giving the significantly slower growth of the left hemisphere when compared to the right hemisphere in utero, particularly in males, Geschwind hypothesized that testosterone preferentially affected the growth of the left hemisphere[16]. Animal studies have also

identified a high-density of androgen-sensitive receptors in multiple key cortical areas in both the left and right hemispheres[17, 18]. In addition, reports from animal samples have demonstrated a sex-specific effect of testosterone in which testosterone appears to protect against neuronal loss associated with developmental remodeling in females[17, 18].

On the other hand, the critical time period during which testosterone would exert its effect on cortical growth remains a matter of debate. While some evidence suggests that organizational effects of testosterone on the central nervous system are already apparent as early as in utero, more recent reports identify later organizational effects of testosterone during puberty and adolescence, and possibly even into young adulthood[1-14]. In addition, puberty coincides with both (1) sex-specific significant increases in testosterone levels and (2) increased differentiation between sexes in cognitive skills such as verbal memory and fine motor dexterity, suggesting this is an important time period when changes in testosterone levels could affect brain development, particularly cortical maturation and the subsequent sex-related differentiation in specific cognitive abilities[19-20].

Testosterone is therefore thought to play a central role in pubertal developmental mechanisms underlying sex-related trajectories of grey matter growth observed in children and adolescents. However, due the limitations of the current literature, the exact nature of testosterone-related sex-specific trajectories in cortical growth, timing of these effects throughout adolescence and brain regions most sensitive to testosterone, remain unclear.

Sex differences in cortical grey matter development

Several structural MRI studies have suggested sex-specific growth trajectories of cortical grey matter in children and adolescents[13, 21-36]. Initial reports emphasized an inverted U-shaped trajectory for grey matter in most regions, and suggested a sex-related age difference, with frontal grey matter and parietal grey matter matter peaking earlier in females (10.2-11 yo (yo)) than males (11.8-12.1 yo), and a possible earlier peak for males in growth of temporal grey matter in males (16.2 yo) compared to females (16.7 yo)[30].

On the other hand, the Brain Development Cooperative Group recently reported results from a large, representative community sample of children and adolescents from 4-18 years old which forms the parent sample that this study draws from[12]. Contrary to several earlier reports, they found fewer sex differences after correcting for total brain volume[12]. Only the relative volume of occipital grey matter differed by sex, being larger in males. Frontal grey matter did not differ by sex, which stands in disagreement with one report where frontal grey matter was greater in females[13]. In addition, modeling grey matter trajectories across the broader age range in the total cross-sectional sample, they found most grey matter measures to be best described by linear functions, not curvilinear[12]. However, females did show greater curvilinearity of grey matter by age than males, especially in frontal, parietal, and temporal areas[12]. Regardless, grey matter decreased progressively across the entire age range from 4-18 years[12]. Declines in grey matter were more prominent and occurred earlier in occipital and parietal lobes[12].

Testosterone and cortical grey matter: cross-sectional associations

While trajectories of grey matter growth have been well-defined in children and adolescents, mechanisms underlying this sexual differentiation are still unclear, and the specific effects of testosterone remain to be clarified. Among studies looking directly at the relationship between testosterone and grey matter development, Paus et al. found that increasing testosterone levels correlated with relative decreases in total relative grey matter volumes in males in a cross-sectional sample of 419 adolescents, 12-18 yo[7]. No regional effects were described, and no associations of testosterone with grey matter in females were observed[7]. Bramen et al. studied a cross-sectional sample of 80 subjects restricted to the narrow age range of 10-14 years and found no associations between testosterone and overall grey matter in boys[8]. They observed decreased total grey matter in girls with increasing levels of testosterone in this sample; however, no corrections for multiple comparisons were applied, and no regional associations of testosterone with grey matter volumes were identified despite multiple analyses[8]. In another cross-sectional sample of 46 children 8-15 yo, Neufang et al. found a negative association between testosterone and left parietal volumes, including the left precuneus and left superior parietal gyrus, and reported a significant 'sex by testosterone' interaction with boys showing a greater effect of testosterone on grey matter[9]. In another cross-sectional sample of 34 young adults with a mean age of 26.6 yo, Witte et al. reported a negative association between grey matter in the left inferior frontal gyrus and testosterone in both men and women[10]. On the other hand, surprisingly, Peper et al. found a male-specific

positive correlation between overall grey matter volume and testosterone levels in a cross-sectional sample of 78 children 10-15 yo; yet no correlation was seen after correcting for total brain volume[11].

In summary, initial examinations have found changes in overall or lobar cortical grey matter associated with testosterone levels, and have been mostly limited to adolescent samples. Results have varied considerably, although within the adolescent age epoch and young adulthood, increasing testosterone levels appear to be associated with decreases in grey matter volumes. The association has been inconsistent between males and females, and limited regional effects have been detected. Discrepant findings in the current literature are most likely due to the differences between studies in the inclusion of various age groups and sampling methods, with most using samples of convenience or regional community-based advertisement.

Of note, no study up to now has examined testosterone-related associations with grey matter in a longitudinal sample, or included a broader age range of younger, pre-adrenarchal subjects. Furthermore, despite preclinical observations of protective effects of androgens in females, no positive associations between testosterone and grey matter have been reported to date. Larger, representative, longitudinal population-based samples across broader age ranges are necessary to discern whether robust relationships between testosterone and grey matter do in fact exist, and to map the age-, sex-, and regional trajectories of those associations. Moreover, given developments in computational neuroanatomy that have now made possible improved MRI-based quantification of

cortical thickness, it is timely to revisit associations between testosterone and more specific brain regions.

Functional testosterone-related activation patterns

In contrast to the structural MRI literature, functional MRI studies have identified testosterone-related activation patterns in more localized brain areas[1, 4-6, 14, 37-40]. However, methodological heterogeneity –variable testosterone doses and cognitive tests- preclude direct comparisons and have led to a variety of findings that are difficult to synthesize. Some fMRI studies found non-specific, generalized increase in activation following testosterone administration[3, 41]. The majority of studies, however, demonstrate increased regional activation with high testosterone levels or testosterone administration[1, 4-6, 14, 37-40].

In adult men, a positron emission tomography study showed associations between peripheral free testosterone and increased regional cerebral blood flow (rCBF) in the cingulate, parahippocampal and right inferior frontal gyri while total testosterone levels were associated with increased rCBF in the left inferior frontal cortex[37]. In a single photon emission tomography (SPECT) study of 7 hygonadal men, testosterone treatment led to increased regional brain perfusion in the superior frontal gyrus and midcingulate gyrus as well as improved cognition[4]. In elderly men, Kumano et al reviewed the evidence for testosterone replacement and found evidence for testosterone-related activation of the superior frontal gyrus and the dorsal anterior cingulate gyrus[1].

In young adult women, testosterone administration is associated with increased activation in the thalamo-cingulate region and insula[6]. In addition, a fluoro-deoxy-glucose positron emission tomography study of 14 anorexic women and 20 controls showed that testosterone replacement led to increased metabolism in the anterior and posterior cingulate cortex, premotor cortex and right parietal lobe[5]. A mixed-gender study of 24 adult men and women also reported testosterone levels to be associated with left prefrontal activation on fMRI during a semantic task[14].

In contrast to the increased activation associated with testosterone reported in the above studies, a study of 34 adults (17 males and 17 females) as well as a study of 27 premenopausal women found *decreased* regional activation in the orbitofrontal cortex with higher testosterone levels and testosterone administration, respectively[42-43].

In summary, most studies demonstrate increased regional activation in the superior and inferior frontal gyri, parahippocampal gyrus, anterior/mid/posterior cingulate gyrus, insular and sensorimotor cortices as well as decreased regional activation in the orbitofrontal cortex with high testosterone levels or testosterone administration[1, 4-6, 14, 37-40].

Objectives and hypothesis

The current literature suggests that grey matter changes during childhood and adolescence may follow pubertally-driven sexual maturation patterns, through the effects of testosterone levels on the central nervous system. In the present study, we examined testosterone-related longitudinal changes in cortical thickness in developmentally healthy children from 4 to 22 years of age using the NIH MRI Study of Normal Brain Development database[44]. Based on the aforementioned structural and functional neuroimaging studies, we hypothesized that, while cortical thickness may be globally associated with testosterone levels, localized effects of testosterone will be more pronounced and will likely affect cortical thickness of the cingulate, parahippocampal, sensorimotor, insular, prefrontal and orbitofrontal cortices. In light of the alleged protective effects of testosterone on cortical development in females, we also hypothesized that testosterone may demonstrate positive associations with cortical thickness in females. Finally, given predicted differential effects of testosterone on hemispheric lateralization, we hypothesized that testosterone levels would tend to affect cortical maturation in the left hemisphere, particularly in males.

METHODOLOGY

Sampling and Recruitment

The National Institutes of Health (NIH) MRI Study of Normal Brain Development is a multi-site project providing a normative database to characterize healthy brain maturation in relationship to behavior[44]. Subjects were recruited across the United States with a population-based sampling method seeking to minimize biases of selection and to achieve a representative sample in terms of income level and composition by race and ethnicity based on the US Census[45]. Informed consent from parents and child assent were obtained for all subjects[44-45]. The Objective 1 database (release 4.0) used for this study included 433 healthy children from 4 to 22 yo (226 females, 207 males) who underwent extensive cognitive, neuropsychological and behavioral testing along with repeated MRI brain imaging every 2 years, with a maximum of 3 scans over 4 years (visit 1 at study onset, visit 2 two years later and visit 3 four years after study onset). In order to limit the sample to developmentally healthy children, rigorous exclusion criteria were applied, including current or past treatment for a DSM-IV axis 1 psychiatric disorders, learning disabilities, evidence of significant axis I disorders (schizophrenia, bipolar affective disorder, psychotic disorder, major depression, Obsessive-Compulsive Disorder, Tourette Syndrome) on structured parent or child interview (DICA), family history of major axis 1 disorder (schizophrenia, bipolar affective disorder, psychotic disorder, major depression, Obsessive-Compulsive Disorder, Tourette Syndrome), family history of inherited neurological disorder or mental retardation due to non-traumatic events, abnormality on

neurological examination, gestational age at birth <37 weeks or >42 weeks, intra-uterine exposure to substances (alcohol, drugs of abuse, nicotine) known or highly suspected to alter brain structure or function. After strict quality control of MRI data (see 'automated image processing' section), the sample for this study included 282 subjects (155 females, 127 males), with a total of 469 MRI scans (270 scans from female subjects, 199 scans from male subjects).

MRI protocol

In order to collect data within the time limitations for this age range and to allow automated morphometric analysis, 30-45 minutes of data acquisition was allocated, with 1 mm in-plane resolution, 1-2 mm slice thickness, whole brain coverage and multiple contrasts (T1W, T2W and PDW)[44]. A 3D T1-weighted spoiled gradient recalled (SPGR) echo sequence was selected.

Automated Image Processing

Quality controlled native MR images were processed through the CIVET automated pipeline (version 1.1.9, 2006)[46]. This pipeline includes the CLASP algorithm for generating cortical thickness measurements at 40,962 vertices per hemisphere[46-50]. Cortical thickness is calculated as the distance at each vertex between the 'outer CSF-grey matter' and 'grey matter-white matter' interfaces, whereas the surface area is defined as the mid-surface of the native triangular area at each vertex [47-49]. Thus this methodology

allows the distinction between cortical thickness itself and surface area, as opposed to voxel-based morphometry. In addition, cortical thickness measured in this way at each vertex takes into account the vertical alignment of cortical columns and allows for more precise measurement of cortical grey matter than voxel-based morphometry when two adjacent gyri may be included in the same voxel and appear fused in the image. The cellular mechanisms that underlie changes in cortical thickness have yet to be clarified, but animal studies suggest that a change in arborisation and size of the neurons, rather than a change in the number of neurons, may be the underlying mechanism.

A stringent manual quality control by two independent investigators of the native cortical thickness images of each subject was implemented, with an inter-rater reliability of over 0.9[51]. The strict quality control procedure excluded MRI scans from: (1) subjects with fallback protocols, who could not remain in the scanner for an extended period of time and had to undergo faster MRI image acquisition, resulting in a degree of resolution too low for an adequate estimation of cortical thickness (2) subjects with severe motion abnormalities resulting in image processing failures (3) subjects with strong field inhomogeneity in the image that could not be adequately corrected (4) subjects with no available measurement of salivary testosterone levels or inadequate sampling.

Testosterone collection

Over the course of 4 years, during each of their 3 visits, children provided two separate 1–3 cm^3 samples of saliva, collected at two time points during the day, which were

assayed by published radioimmunoassays (RIA) methods for testosterone[52]. Relative to other gonadal hormones, peripheral levels of testosterone may be particularly useful in efforts to assess its effect on the central nervous system, given its tendency to cross the blood-brain barrier, in contrast to estradiol which is mostly bound to alpha-fetoprotein[15]. In particular, salivary testosterone measures represent free, biologically available hormone, which may be more relevant to studies of brain-hormone associations during development. Although the majority of samples were obtained between mid-morning to early afternoon, a period of reduced diurnal fluctuation for testosterone, variability of collection times led to the selection of the earliest sample of testosterone for each subject in order to maximize data homogeneity[52].

Pubertal measures

The Pubertal Development Scale (PDS) was administered to all subjects in the restricted sample[53]. It consists of 5 items on a 4 point ordinal scale for each gender and has been shown to have good reliability (coefficient alpha: 0.77) and validity (r^2=0.61-0.67) compared to physical examination[53]. Following the method previously used by Wichstrom et al., we computed a puberty variable consisting of 5 stages, based on the sum of scores for each of the 5 items of the PDS divided by 5[54]:

1. Prepubertal = 0 to 1.7
2. Early Pubertal = 1.8 – 2.4
3. Midpubertal = 2.5 – 3
4. Late Pubertal = 3.1 – 3.6

5. Post pubertal = 3.7 – 4

In addition, we also defined a dichotomous puberty variable for pre- and post-pubertal stages, with stages 1 and 2 representing pre-pubertal subjects and stages 3, 4 and 5 representing post-pubertal subjects in order to analyze data for these two groups independently.

Statistical Analyses

Statistical analyses were implemented using SurfStat, a statistical toolbox created for MATLAB 7 by Dr. Keith Worsley (http://www.math.mcgill.ca/keith/surfstat/). Each subject's absolute native-space cortical thickness and surface area were linearly regressed against the natural logarithm of testosterone (ln_testo) at each cortical point. A whole-brain correction, using random field theory (RFT) with $p<0.05$, was used in all analyses to account for multiple comparisons[55].

To account for repeated MRI scans in each subject, a mixed effects linear model was applied to all subjects, controlling for age, total brain volume, sex and testosterone collection time. Of note, because testosterone values were not normally distributed, a natural logarithmic transformation was used to normalize the data for analysis[56]. The interactions between testosterone, age and sex was tested using a full model with testosterone*sex*age interaction as the highest order term.

To illustrate differences in the association of testosterone with cortical thickness between subjects at different ages, linear mixed effects models were run at different centered ages. This approach is similar to other published methods, and uses a modified age term to examine group differences at each age based on values estimated from developmental curves modeled on acquired data for all subjects[57].

To assess the impact of puberty as defined by the PDS, we replaced the testosterone variable in the model by an ordinal puberty variable consisting of 5 pubertal stages. We also ran separate analyses with both the testosterone and puberty variables to determine the effect of each predictor variable while controlling for the other.

To assess the impact of handedness, we ran separate analyses controlling for handedness in addition to all the aforementioned control variables and using the full model with testosterone*sex*age interaction as the highest order term.

RESULTS

Demographics

Comparisons between demographic characteristics of the initial NIH cohort (n=433) and the resulting sample after strict quality control for cortical thickness (n=282, 127 males, 155 females) are shown in **Table 1**. Mean age was higher in the restricted sample (mean 13.1 years old +/- 3.6; range 4-22 years old) as opposed to the initial NIH cohort (mean 11.8 years old +/- 4.1; range 4.6 to 22.3 years old), as younger age was associated with higher rates of image processing failure. Consequently, mean testosterone levels were also slightly higher in the restricted sample (mean 60.7 ng/dl +/- 59.1; range 5.7 to 574.4 ng/dl) than in the initial NIH cohort (mean 54.3 ng/dl +/- 56.4; range 4.5 to 574.4 ng/dl) though the range of values was similar in both groups. **Table 2** shows the demographic characteristics of males and females in the restricted sample. As expected, males (mean 71.6 ng/dl +/- 65.4) had significantly higher average testosterone levels compared to females (mean 52.7 ng/dl +/- 52.8), but there were no other significant differences between the two sexes in our sample, including for handedness.

Testosterone and cortical thickness: complete sample and subgroup analyses

As outlined in **Figure 1**, there was a negative association between testosterone and cortical thickness in the left posterior cingulate cortex (Brodmann area 30) for males and females of all age groups (282 scans, 469 scans). This association was driven by post-

pubertal males (56 subjects, 77 scans), who showed negative associations between testosterone and cortical thickness involving the left posterior cingulate gyrus as well as the adjacent precuneus area (Brodmann areas 23, 30, 31). In post-pubertal males, there was also a trend for a negative association between testosterone and cortical thickness in the left dorsolateral prefrontal cortex (Brodmann area 46). In pre-pubertal females, there was a trend for a positive association between testosterone and cortical thickness in the right somatosensory cortex. However, these trends did not survive correction with random field theory.

Age effect in the complete sample

Analyses by age group of the whole sample (282 scans, 469 scans) revealed an 'age by testosterone' interaction on cortical thickness. Testosterone-related associations with cortical thickness thus varied across the age range.

More specifically, in the right hemisphere, there was a positive association between testosterone levels and cortical thickness in the somatosensory cortex (Brodmann areas 1, 2, 3) from ages 4 to 7, which later reversed to a negative association between testosterone levels and cortical thickness of the same area between ages 17 to 22 (**Figure 2**).

In the left hemisphere, there was a negative association between testosterone and cortical thickness in the posterior cingulate cortex (Brodmann areas 23, 30, 31) and the dorsolateral prefrontal cortex (Brodmann area 46) from ages 14 to 22, as well as a

negative association between testosterone and cortical thickness of the anterior cingulate (Brodmann areas 24 and 33) and parahippocampal gyri (specifically the perirhinal cortex, Brodmann area 35) from ages 21 to 22 (**Figure 3**). There were no significant associations between testosterone and cortical thickness in the complete sample from ages 8 to 13.

Age effect in males

In males (127 subjects, 199 scans), as shown in **Figure 4**, there was a negative association between cortical thickness and testosterone levels in the left posterior cingulate cortex (Brodmann areas 23, 30), starting at age 14, progressing as children age to include the left precuneus (Brodmann area 31) between ages 16 to 19 and the left anterior cingulate cortex (Brodmann areas 24 and 33) from ages 20 to 22. In addition, there was a negative association between testosterone levels and cortical thickness in the left dorsolateral prefrontal cortex (Brodmann area 46) from ages 16 to 21. There were no significant associations between cortical thickness and testosterone levels between the ages of 4 and 14.

Age effect in females

In females (155 subjects, 270 scans), as outlined in **Figure 5**, there was a positive association between testosterone levels and cortical thickness in the right somatosensory cortex (Brodmann areas 1,2,3) from ages 5 to 8, a relationship that later reversed to a

negative association in the same area from ages 20 to 22. There were no significant associations between testosterone levels and cortical thickness between ages 9 and 19.

Sex and age interactions

In addition to the 'age by testosterone' interaction described above for the complete sample, as well as for males and females separately, the 'sex by testosterone' and 'sex by age' interactions on cortical thickness were also significant for several brain regions of both the left and right hemispheres (**Figure 6 and Figure 7**). We therefore examined the triple 'sex by age by testosterone' interaction on cortical thickness for the complete sample, and found that it also involved multiple regions across the two hemispheres (**Figure 8**). Looking specifically at post-pubertal subjects, we found that the 'sex by age by testosterone' interaction involved even more widespread brain areas than in the full sample (**Figure 9**). In order to better understand and visualize the triple interaction, it was decomposed by sex for a few key areas in **Figure 10.**

Of note, while specific and restricted regional associations were described above for males and females separately, 'sex by age by testosterone' interaction analyses suggest significant and diffuse differences between males and females in testosterone-related cortical maturation across childhood and adolescence. However, when males and females were tested separately, thereby resulting in a decrease in power, testosterone-related cortical maturation in many of these brain regions is not striking enough to survive correction with random field theory.

Testosterone-related lateralization in males and females

The association between testosterone and cortical thickness of the left versus the right hemisphere differed in males and females. Males showed significant testosterone-related thinning of the left dorsolateral and ventral prefrontal cortex when compared to the same regions of the right hemisphere (**Figure 11**), while females showed testosterone-related thickening of the right compared to the left somatosensory cortex (**Figure 12**).

Pubertal stages

When using a puberty variable made of 5 defined pubertal stages based on the Pubertal Developmental Scale (PDS) to replace testosterone in our analyses, we obtained similar results to those outlined above, with a decreased level of significance. Analyses with both the testosterone and puberty variables revealed that the association between testosterone and cortical thickness remained significant after controlling for pubertal stages; however, the pubertal effect did not remain significant after controlling for testosterone levels.

Handedness

Adding handedness as a control variable to the above analyses, there were no significant differences in the findings outlined above.

DISCUSSION

Our study identified novel interactions between testosterone and developmental changes in cortical thickness. One of several unique findings was evidence for positive effects on cortical thickness during childhood, as well as replication of some, but not all, prior reports of negative associations of testosterone with cortical thickness during mid- to late adolescence. These relationships may help to explain the long-hypothesized influences of gonadal hormones, in this case testosterone, on shifts in behaviors, psychopathology, and cognitive advantages. Despite controlling for multiple comparisons and variables known to covary with cortical thickness, quite remarkably the discrete regions where testosterone appears to interact during development with cortical thickness are areas identified in part to comprise networks subserving cognitive control. Our findings also underscore the protracted nature of developmental changes in cortical thickness associated with testosterone levels extending into the third decade of life.

Specifically, we found complex interactions varying according to sex and age between testosterone and cortical thickness of widespread areas in both hemispheres, which may explain inconsistencies between previous studies with a more restricted age span. However, when males and females were tested separately, more regional effects emerged. Post-pubertal males showed a negative association between cortical thickness and testosterone levels in the left hemisphere, involving the posterior cingulate cortex, precuneus and the dorsolateral prefrontal cortex and later the anterior cingulate gyrus. Early pre-pubertal females showed a positive association between cortical thickness and

testosterone levels in the right hemisphere, involving the somatosensory cortex, a relationship that was reversed in late post-pubertal females with a negative association in the same area. To our knowledge, there are no prior reports identifying positive relationships between testosterone and cortical thickness; however, such a relationship may be consistent with observations in developing animals of a neuroprotective influence of testosterone in females from developmentally programmed apoptosis[18].

We also explored whether parallel relationships with cortical thickness could be discerned when pubertal stages were used as a proxy for hormonal influences. There were similar, but less significant associations between cortical thickness and pubertal staging as defined by the PDS, when compared to results based on testosterone levels. Therefore, testosterone levels and the PDS appear to reflect similar processes, but the measurement of testosterone levels as an index of increasing pubertal maturity was associated with more robust interactions with cortical thickness as opposed to using pubertal status as defined by the PDS.

Sex differences in cortical maturation: impact of regional associations between testosterone and cortical thickness

Consistent with the Brain Development Cooperative Group's recent report from the parent sample, we found cortical maturation to be best described overall by negative linear functions between testosterone and cortical thickness across the entire age range, particularly for males[12]. Interestingly, in females there was a positive, followed by a

negative association between testosterone and cortical thickness in the right somatosensory cortex that is also consistent with the report from the parent sample, which showed better fit for curvilinear functions in females[12]. Declines in grey matter which were found to be more prominent in occipital and parietal lobes in the parent sample were also found in this study to be associated with increasing testosterone levels[12]. Finally, our results parallel previous reports of greater frontal grey matter in females, as we found significant associations between testosterone levels and a thinner prefrontal cortex in males[13]. Thus testosterone appears to play an important role in developmental cortical maturation and accounts for several of the sex differences in GM growth across childhood and adolescence.

Functional sex differences: impact of regional associations of testosterone and cortical thickness

Higher testosterone levels and testosterone administration appear to be associated with increased metabolism/cerebral blood flow in the cingulate, insular, superior/inferior frontal, parahippocampal gyri and the parietal sensorimotor cortex in adolescent and adult samples[1, 4-6, 14, 37]. Taken together with the results of the present study, this suggests that testosterone-related activation of brain regions is associated to a thinner cortex in specific brain regions (parahippocampal gyrus in both males and females; cingulate and dorsolateral prefrontal cortices in males; somatosensory cortex in females). Findings are also consistent with observations of a linear, age-related increase in activation of parieto-

occipital regions and the precuneus throughout puberty and young adulthood, related to mental rotation tasks, in adolescent and adult men age 13 to 28[58].

On the other hand, as opposed to the localized effects of testosterone mentioned previously, reports of widespread activation throughout the cortex with the administration of testosterone are also consistent with our findings of sex differences in testosterone-related cortical maturation in multiple brain regions identified by the 'sex by age by testosterone' interaction[3, 41]. However, these diffuse effects of testosterone on cortical thickness fell short of significance when males and females were tested separately, and therefore appear to be less pronounced than stronger regional effects already described.

Of note, in this study we did not find an association between testosterone levels and the insular and orbitofrontal cortices. This may be partly accounted for by methodological limitations in the measurement of insular and orbitofrontal cortical thickness, as the high degree of gyrification in this former area and magnetic distortions in the latter preclude precise estimation of cortical thickness with the current automated algorithm. In addition, regarding the orbitofrontal cortex, previous functional studies have found *decreased* rather than increased activation with high testosterone levels or testosterone administration; this may explain the lack of association in this area given its different relationship to testosterone compared to activational effects of testosterone in other regions of interest. Finally, the association between testosterone and the parahippocampal gyrus, found in the complete sample, was not found when males and females were tested separately, which may reflect a relative decrease in power in the latter analyses.

Sex differences in cognition: impact of regional associations of testosterone and cortical thickness

In this study, we found the posterior cingulate cortex, precuneus, anterior cingulate cortex, dorsolateral cortex and somatosensory cortex to be most significantly associated to testosterone levels and therefore possibly most sensitive to the effects of testosterone. Concerning functions previously ascribed to these brain regions, the posterior cingulate cortex has been shown to be involved in spatial orientation and episodic memory[59], the precuneus in visuospatial tasks such as mental rotation and block design tasks[60] and the the somatosensory cortex in perceptual sensory functions[61]. On the other hand, the anterior cingulate cortex appears to play a role in attention and emotional regulation[59] while the left dorsolateral cortex has been implicated in executive function, verbal working memory, anxiety and depression[62-63].

In terms of cognitive development throughout puberty, the current literature on sex-specific cognitive differences has shown a pattern of increasing differentiation between sexes across adolescence[15, 64]. Males usually perform better on gross visuospatial tasks such as block design, while females perform better on verbal tests and fine sensorimotor tasks such as the Purdue Pegboard task[15, 20]. It was also noted that in late adolescence, verbal learning in males improved while verbal learning in females declined. In contrast, fine motor dexterity improved in females but declined in males[20].

Consistent with changes in cognition observed across puberty, studies looking

specifically at the association between testosterone and cognition have shown positive associations between testosterone levels and visuospatial skills in both males and females[65-66], while lower levels of testosterone were associated with better verbal fluency in a sample of young women[66]. Lower levels of testosterone were also associated with higher anxiety and attentional problems in boys[67].

Therefore, in light of the current literature, it could be speculated that testosterone-related sex-related differences in cortical thickness in the posterior cingulate and precuneus observed in the current study may play a role in sex-related differentiation of visuospatial skills throughout childhood and adolescence. In turn, sex-specific differences in cortical thickness in the primary somatosensory cortex may be associated with differences in sensory gating/threshold and influence fine motor dexterity as measured by the Purdue Pegboard task. Finally, testosterone-related differences in cortical thickness in the left dorsolateral cortex may be associated with differences in verbal memory and fluency, while testosterone-related effects on the anterior cingulate cortex may be associated to sex-specific differences in attention, and thus may account for the greater vulnerability of males to disorders of cognitive control such as attention-deficit hyperactivity disorder.

Sex differences in hemispheric lateralization: impact of hemispheric associations of testosterone and cortical thickness

Several animal and human studies suggest that sex steroids, in particular testosterone, may play a role in cortical asymmetry and therefore affect cerebral dominance[68]. On the

other hand, evidence for testosterone-related lateralization of cognitive functions is limited by methodological heterogeneity, small sample sizes, hormonal variations and limited reporting about the strength and direction of lateralization[19]. Pfannkuche et al. did not find any significant effects of androgens on language lateralization and handedness in their meta-analysis of 13 human studies[19]. However, 8 of the 13 studies included in this meta-analysis compared controls to subjects exposed to non-physiological testosterone levels *in utero*, such as subjects with congenital adrenal hyperplasia or congenital androgen deficiency[19]. In fact, attention has been mostly focused to date on prenatal testosterone levels rather than peripubertal changes in testosterone[16, 19, 69].

In the present study, we found peripubertal testosterone levels to affect CTh in the left hemisphere more than the right hemisphere in males, while the opposite held true for women, with the right hemisphere being more significantly affected by testosterone levels than the left hemisphere. The same findings persisted when controlling for handedness, supporting the view that manual dominance likely follows a different process from lateralization of cognitive functions such as verbal or visuo-spatial dominance[16]. This relationship between testosterone and respectively, the left hemisphere in males and the right hemisphere in females, was also shown to be age-specific and to evolve throughout puberty. These results suggest that lateralization follows a different pattern in males and females, as well as representing a more dynamic process than previously thought. This is also supported by recent investigation into the inter-hemispheric inhibition of the dominant hemisphere on the non-dominant hemisphere and functional organization within hemispheres, showing that these functional cerebral

asymmetries are sex-specific and sex hormone-dependent, being relatively stable in men and fluctuating in women across the menstrual cycle[70].

In addition, our results are in accordance with Geschwind's theory of lateralization, which predicts testosterone levels to be associated in males with decreased growth in the left hemisphere in specific brain areas. However, while the theory focused on testosterone exposure in utero and early childhood, we found that, in males, peri- and post-pubertal levels of testosterone were significantly associated with decreased cortical thickness in the left hemisphere[16].

Interestingly, a recent theory offers an even more compelling explanation: Lauter's hypothesis of a non-linear association between testosterone and hemispheric growth suggests that relative changes in testosterone exposure may affect both hemispheres differentially, e.g. the left hemisphere affected with moderate changes in testosterone levels, and the right hemisphere affected only with higher levels of exposure[19, 71]. While this theory also focused primarily on prenatal testosterone and did not address within-sex changes in exposure to testosterone, some of our findings are consistent with a non-linear relationship between testosterone and cortical thickness, as testosterone only led to a decrease in cortical thickness in the right hemisphere when it reached higher levels in post-pubertal females (age 20-22) as compared to its potential protective effect on neuronal cell loss in pre-pubertal females with much lower testosterone levels[19, 71]. This hypothesis would also account for differences in testosterone-related cortical maturation between males (associations with the left hemisphere) and females (associations with the

right hemisphere), as males tend to maintain on average higher levels of testosterone than females throughout puberty[71].

Strengths and limitations

Limitations include methodological variations in collection times of salivary testosterone that may have led to decreased sensitivity and specificity of the measured peripheral free testosterone levels, even though we attempted to correct for this by controlling for collection time in subsequent analyses. There was also no data collected on androgen receptor polymorphisms which may affect the degree of cortical growth or cortical thinning, particularly in males[32]. Finally, potential methodological limitations regarding precise estimation of cortical thickness in the insular and orbitofrontal cortices have been already described.

On the other hand, research into testosterone-related effects in the human brain remains scarce, with few structural studies involving pediatric samples. These findings represent the first clear evidence of testosterone-related sex differences in differential cortical growth between the left and right hemispheres in a critical developmental period. Findings stress the importance of looking at changing levels of testosterone across puberty rather than limiting explorations to prenatal testosterone. This study also offers novel insights into (1) the neuroendocrine mechanisms underlying sex differences in the human brain and (2) the development of the brain throughout puberty, as well as suggesting testosterone-related mechanisms underlying (3) sex-specific differentiation of

cognitive abilities throughout childhood and adolescence and (4) sex differences in hemispheric lateralization. Strengths also include the large sample size, the broad age range examined, and the longitudinal design allowing modeling of the relationship between testosterone levels and cortical thickness at each age point.

CONCLUSIONS

There are significant sex- and age-specific associations between testosterone and cortical thickness in the developing brain, consistent with complex and protracted organizational effects. While sex-specific testosterone-related cortical maturation appears to affect widespread areas in both hemispheres, more pronounced regional effects were observed in this study in key cortical regions known to be implicated in visuospatial and sensorimotor skills as well as cognitive control and executive functions. These findings suggest a significant role for testosterone in sex-related differentiation of specific cognitive abilities, as well as risk for disruptive and behavior disorders throughout childhood and adolescence. In addition, both the male-specific association between testosterone and cortical thickness of the left hemisphere and the female-specific association between testosterone and cortical thickness of the right hemisphere support the view that sex differences in hemispheric lateralization progress throughout puberty. Future research should focus on how these testosterone-related structural differences form a neurobiologic substrate that could explain developmental shifts in lateralization of specific cognitive functions.

FIGURES

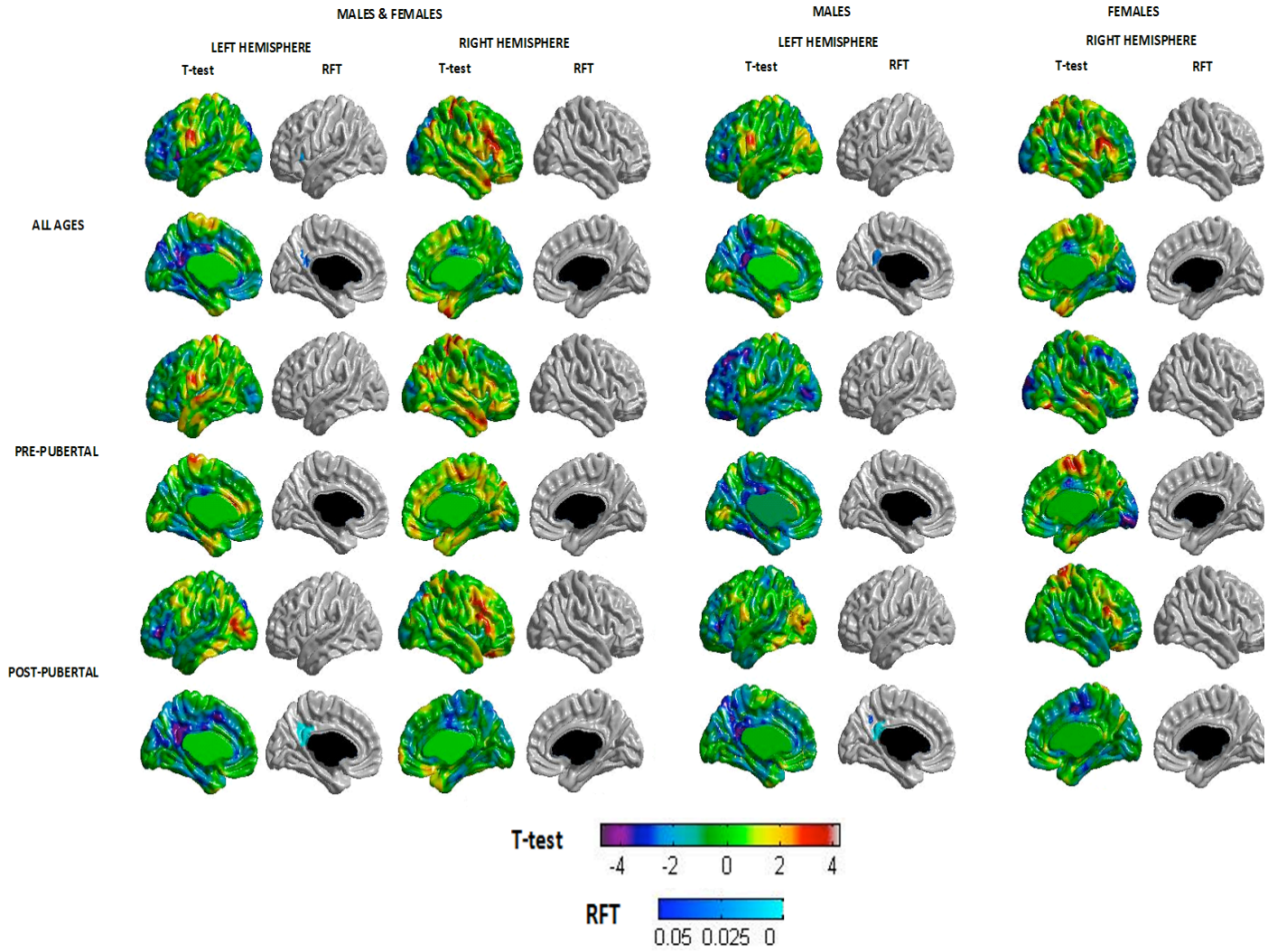

FIGURE 1: Linear associations between testosterone and cortical thickness of the left and right hemispheres, midsagittal and lateral views, for males and females (282 subjects, 469 scans), and males (127 subjects, 199 scans) and females (155 subjects, 270 scans) separately. The figure shows associations for all ages, pre-pubertal and post-pubertal subjects. The scale used for the non-thresholded figures (T-test) pictures the strongest negative associations in purple and the strongest positive associations in red, with the shades in-between representing intermediate associations. The scale used for the figures corrected for multiple comparisons, using random field theory (RFT) pictures the strongest negative associations in light blue.

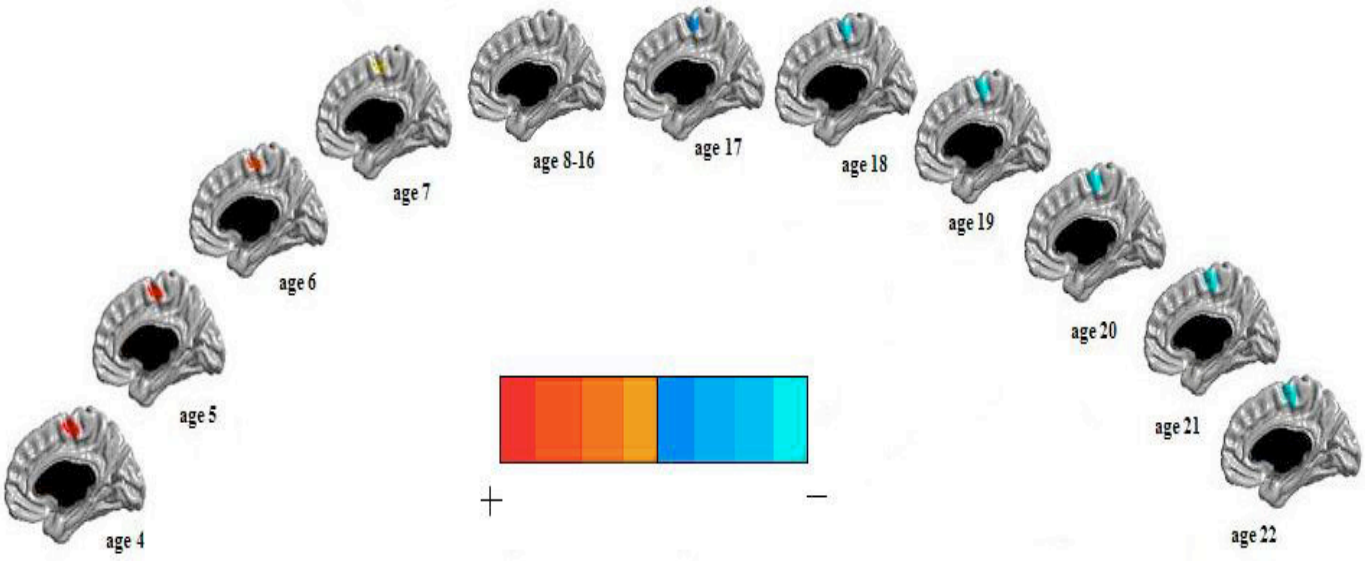

FIGURE 2: Males and females (282 subjects, 469 scans)**: age effect in the right hemisphere,** midsagittal view. The scale used pictures the strongest positive associations in red and the strongest negative associations in light blue, with the shades in-between representing intermediate associations. All associations pictured were significant with $p<0.05$, corrected for multiple comparisons, using RFT.

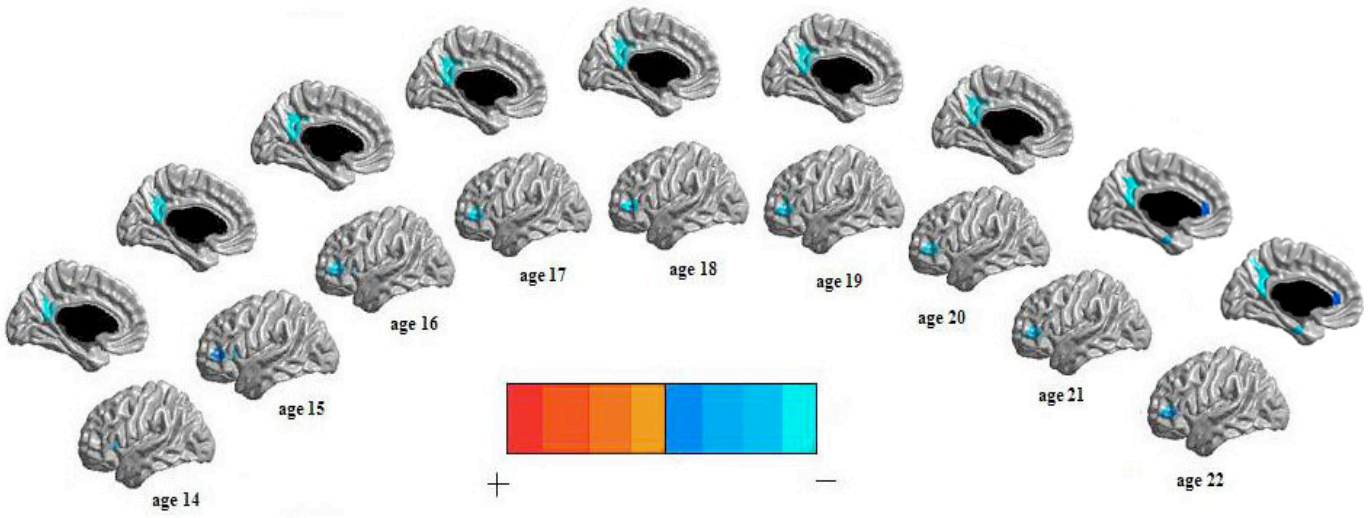

FIGURE 3: Males and females (282 subjects, 469 scans): **age effect in the left hemisphere,** midsagittal and lateral views. Note that ages were truncated to represent only ages 14-22. The scale used pictures the strongest positive associations in red and the strongest negative associations in light blue, with the shades in-between representing intermediate associations. All associations pictured were significant with $p<0.05$, corrected for multiple comparisons, using RFT.

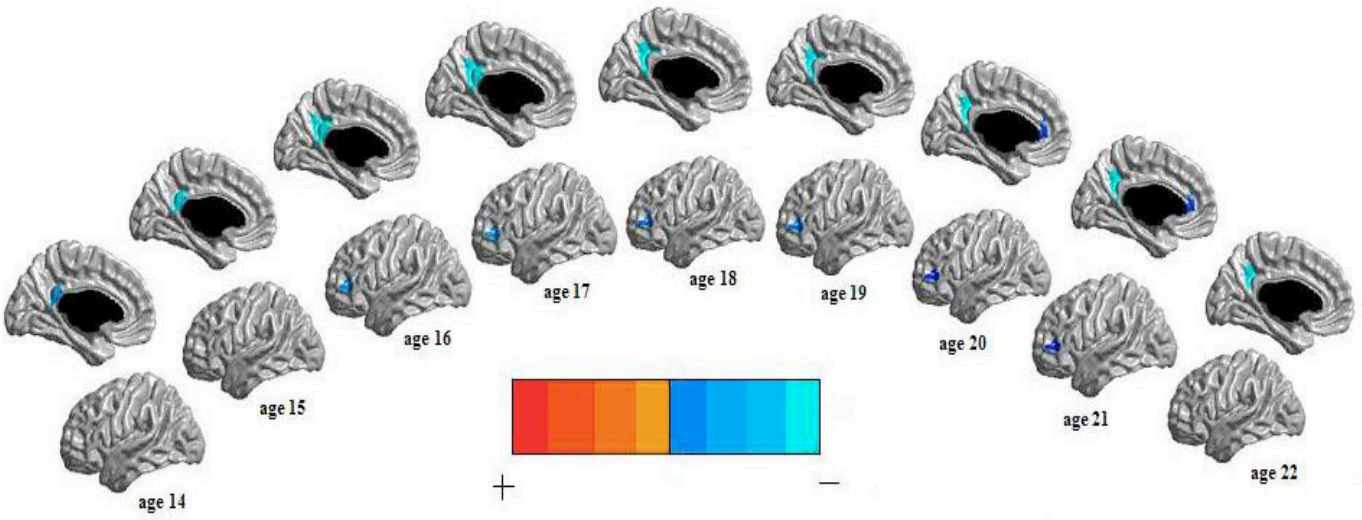

FIGURE 4: Males (127 subjects, 199 scans): **age effect in the left hemisphere**, midsagittal and lateral views. Note that ages were truncated to represent only ages 14-22. The scale used pictures the strongest positive associations in red and the strongest negative associations in light blue, with the shades in-between representing intermediate associations. All associations pictured were significant with $p<0.05$, corrected for multiple comparisons, using RFT.

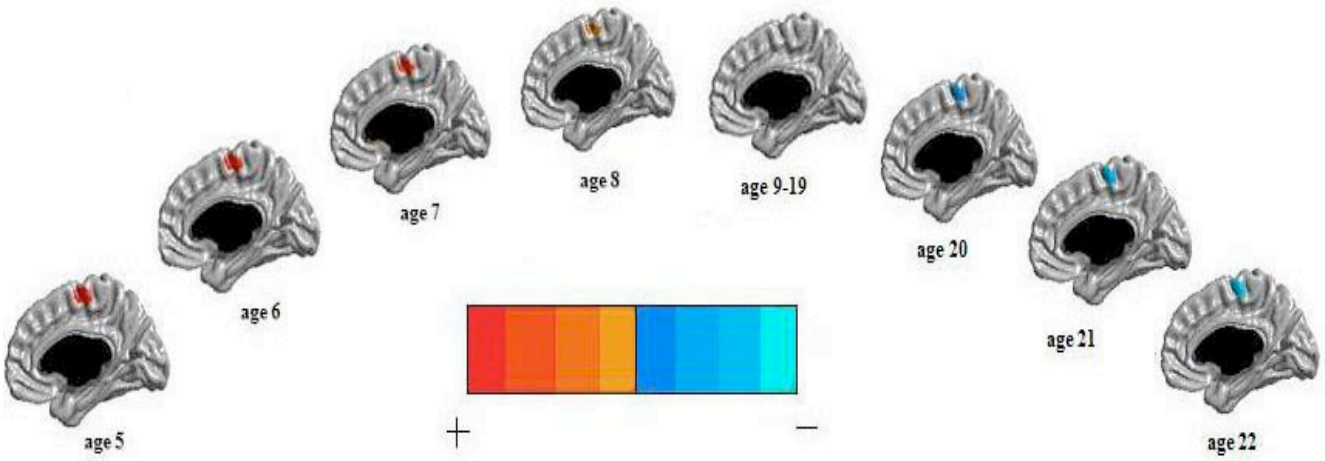

FIGURE 5: Females (155 subjects, 270 scans): **age effect in the right hemisphere**, midsagittal view. The scale used pictures the strongest positive associations in red and the strongest negative associations in light blue, with the shades in-between representing intermediate associations. All associations pictured were significant with $p<0.05$, corrected for multiple comparisons, using RFT.

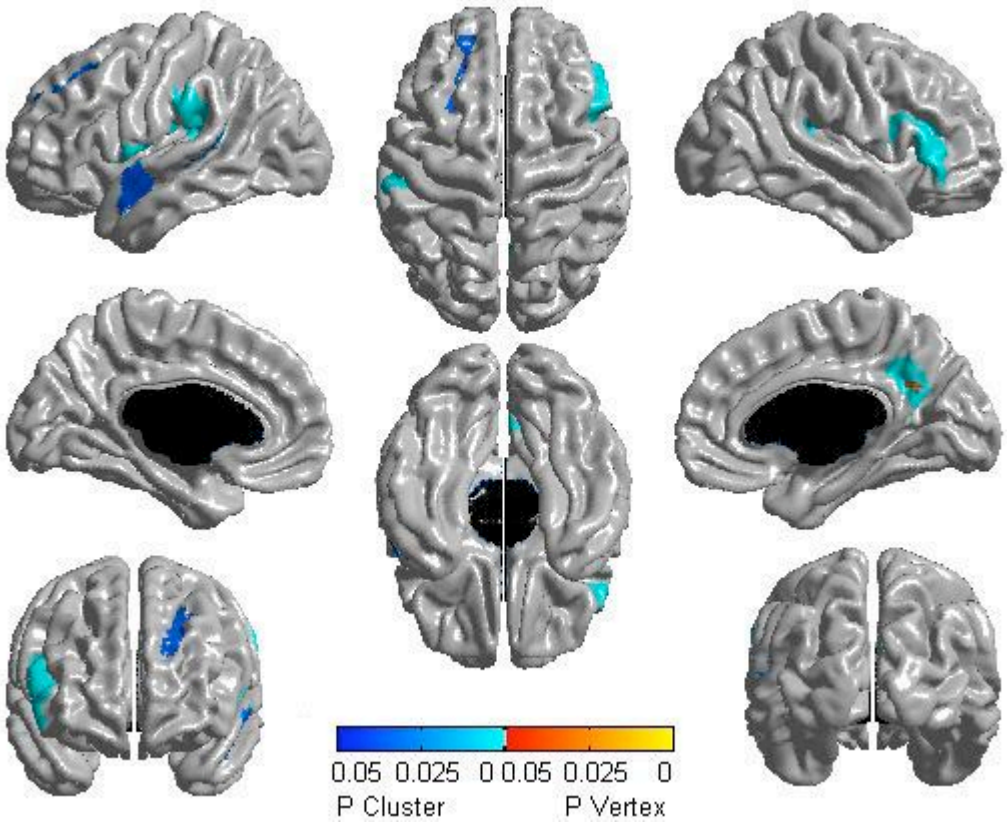

FIGURE 6: 'Sex by testosterone' interaction on cortical thickness in the complete sample (282 subjects, 469 scans)**.** The scale pictures significant interactions in yellow, red and blue, with the interactions in yellow-red being significant at the peak level while those in blue are significant at the cluster level. All associations pictured were significant with $p < 0.05$, corrected for multiple comparisons, using RFT.

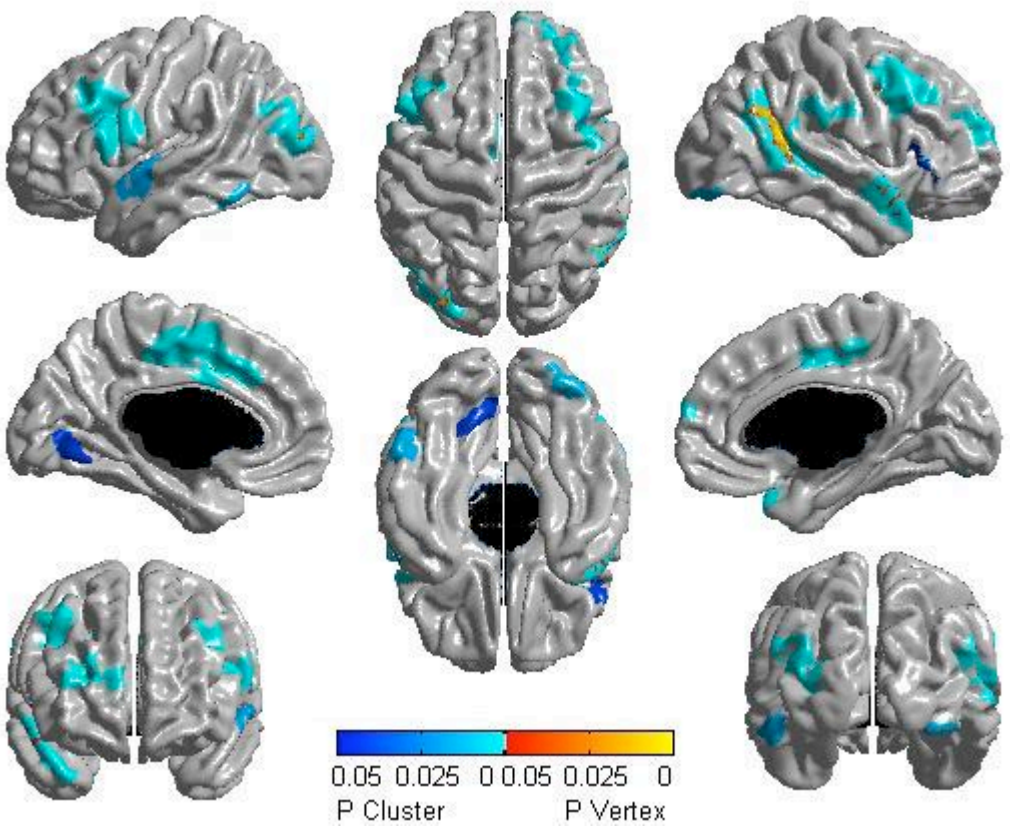

FIGURE 7: 'Sex by age' interaction on cortical thickness in the complete sample (282 subjects, 469 scans)**.** The scale pictures significant interactions in yellow, red and blue, with the interactions in yellow-red being significant at the peak level while those in blue are significant at the cluster level. All associations pictured were significant with $p < 0.05$, corrected for multiple comparisons, using RFT.

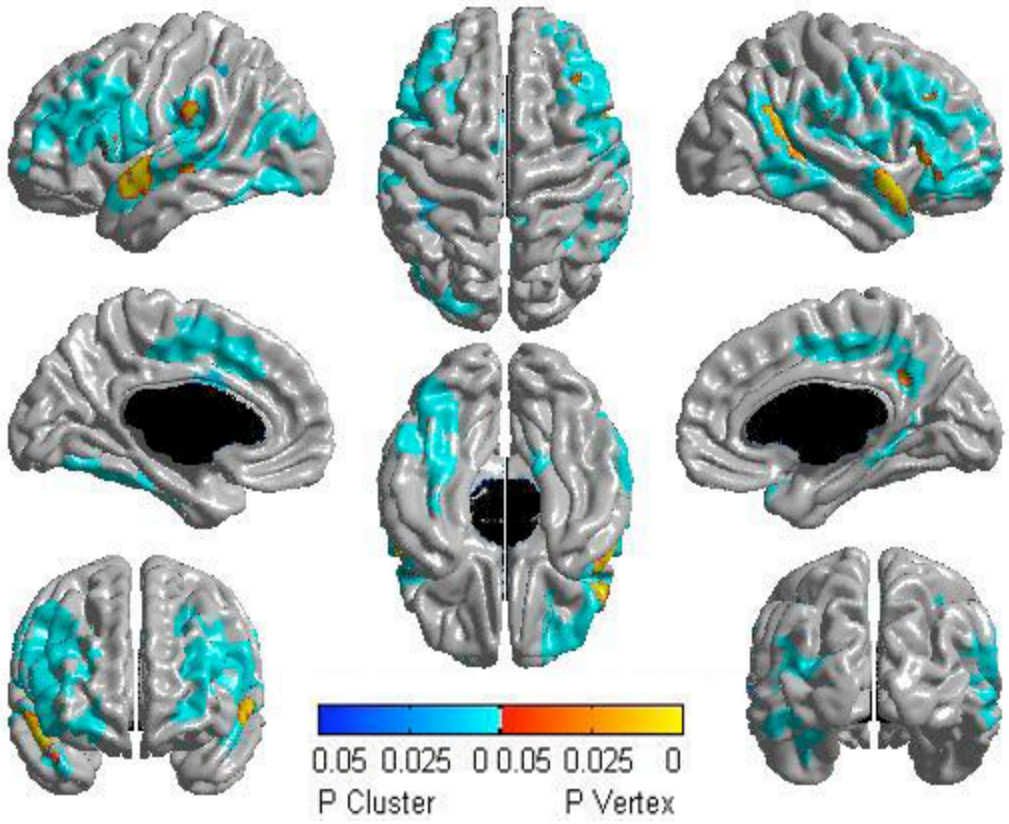

FIGURE 8: 'Sex by age by testosterone' interaction on cortical thickness in the complete sample (282 subjects, 469 scans)**.** The scale used pictures significant interactions in yellow, red, and blue, with the interactions in yellow being most significant. All associations pictured were significant with $p<0.05$, corrected for multiple comparisons, using RFT.

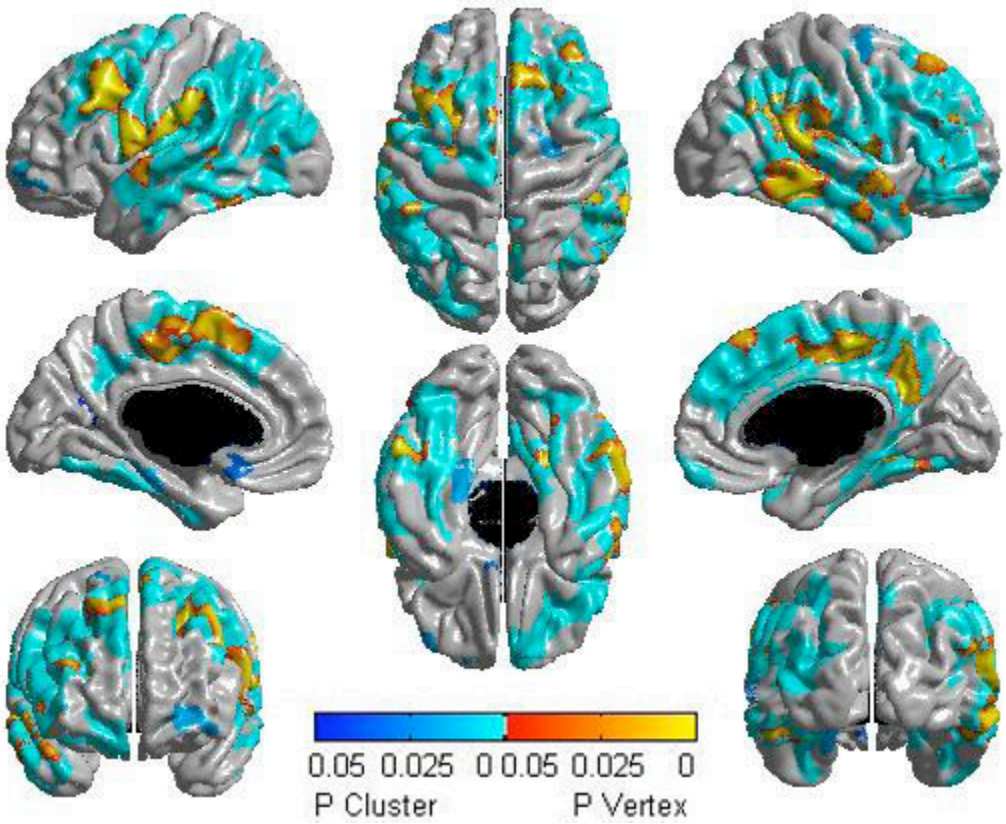

FIGURE 9: 'Sex by age by testosterone' interaction on cortical thickness in post-pubertal subjects (143 subjects, 219 scans)**.** The scale used pictures significant interactions in yellow, red, and blue, with the interactions in yellow being most significant. All associations pictured were significant with $p<0.05$, corrected for multiple comparisons, using RFT.

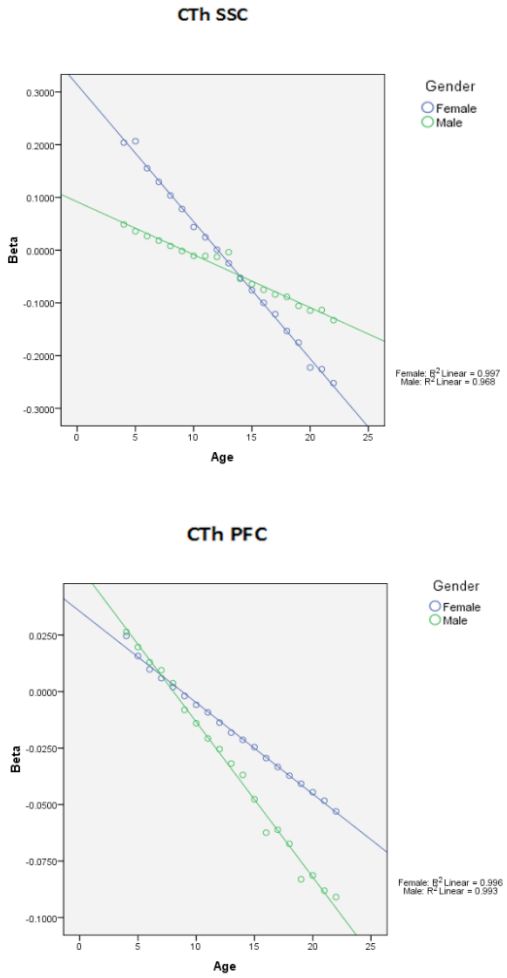

FIGURE 10: Linear regression of beta coefficients for the relationship between testosterone and cortical thickness by age and gender in peak cortical areas. This figure is shown for visualization purposes and represents a decomposition of the triple interaction shown in figure 3 for two selected peaks of statistical significance. Graphs depict the degree of association (beta coefficient=β), at each age, between testosterone and cortical thickness in male and female subjects separately, with a change in β units of testosterone leading to a change in 1 mm of cortical thickness. In these regions, the association between testosterone and cortical thickness becomes more and more negative as subjects age. Further, the slope of the change in association depends on sex and on the specific region involved. SCC: right somatosensory cortex PFC: left dorsolateral prefrontal cortex.

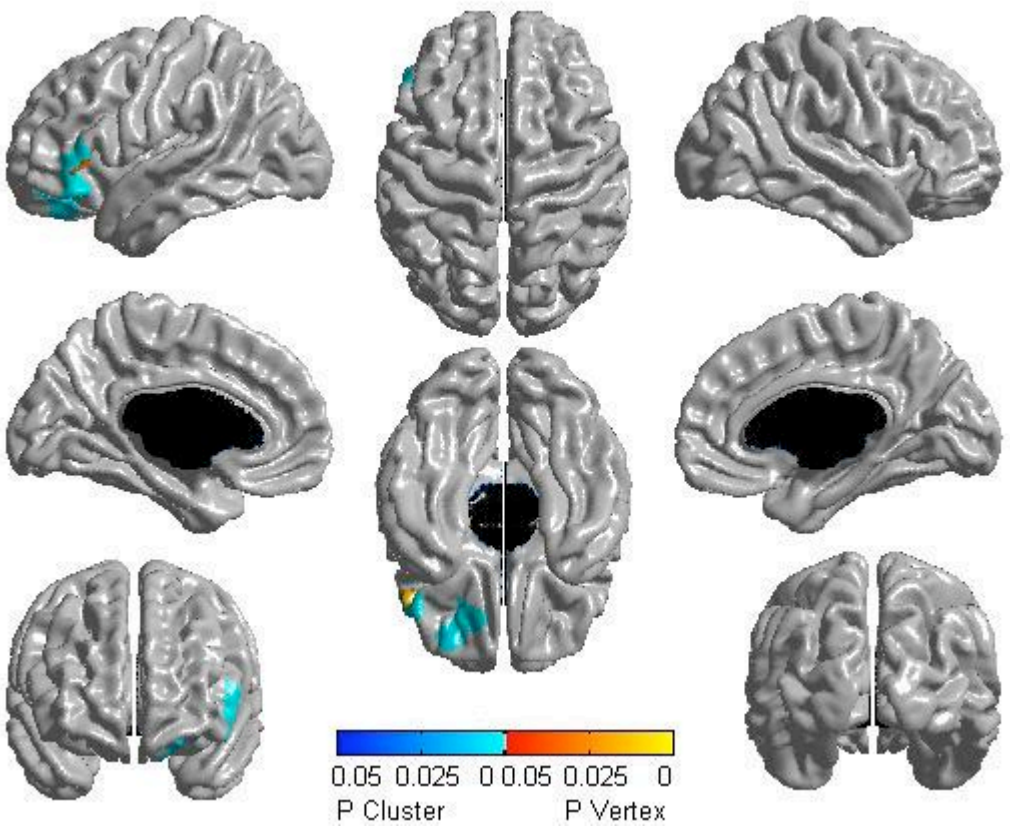

FIGURE 11: Males: testosterone-related lateralization effects (127 subjects, 199 scans). Significant differences between the left and right hemisphere are displayed. The scale pictures significant interactions in yellow, red and blue, with regions in yellow-red being significant at the peak level while those in blue are significant at the cluster level. All associations pictured were significant at $p<0.05$ level, corrected for multiple comparisons, using RFT.

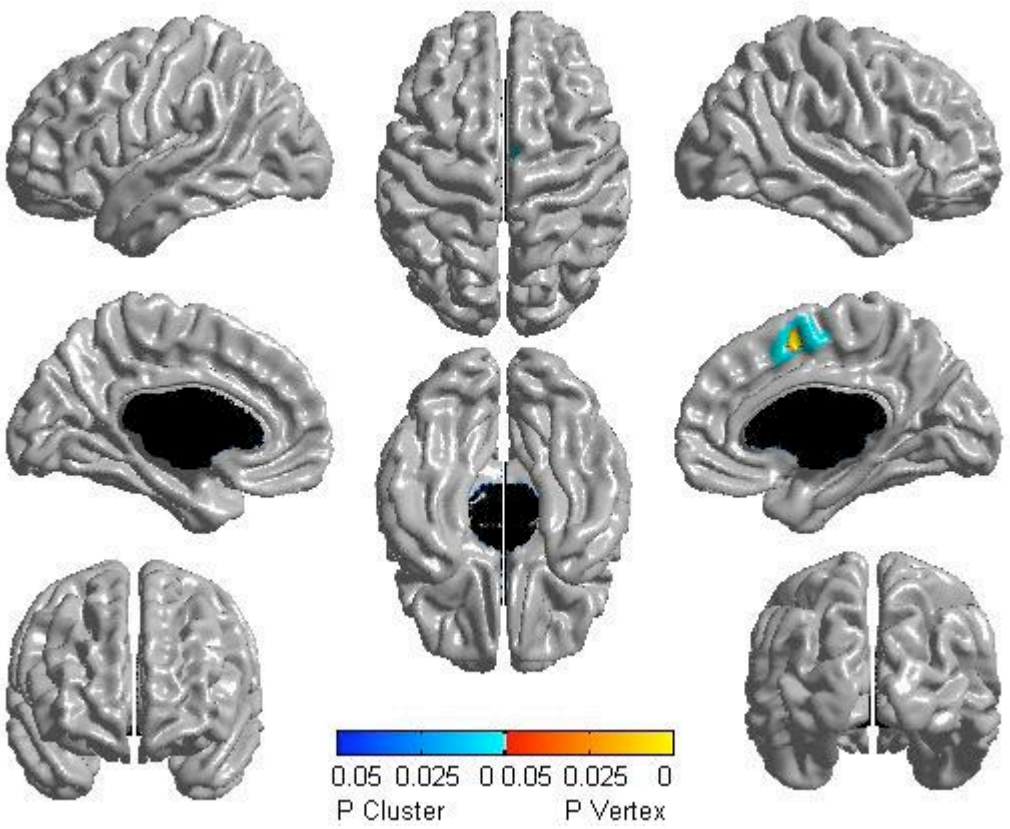

FIGURE 12: Females: testosterone-related lateralization effects (155 subjects, 270 scans). Significant differences between the left and right hemisphere are displayed. The scale pictures significant interactions in yellow, red and blue, with regions in yellow-red being significant at the peak level while those in blue are significant at the cluster level. All associations pictured were significant with $p < 0.05$ level, corrected for multiple comparisons, using RFT.

TABLES

Table 1: Demographic comparisons of the complete NIH Objective 1 cohort (n=433) and restricted quality controlled sample (n=282)

	n=433	**n=282**
Age in years[a]	Range: 4.6 to 22.3 Mean: 11.8 +/- 4.1	Range : 4.9 to 22.3 Mean : 13.1 +/- 3.6 $p<0.0001$
Gender [b]	Females: 226 (52.2%) Males: 207 (47.8%)	Females: 155 (55.0%) Males: 127 (45.0%) $\chi^2=0.86$ $p>0.05$
Handedness[b]	Right: 385 (89.5%) Left:45 (10.5%)	Right: 252 (89.4%) Left: 30 (10.6%) $\chi^2=0.006$ $p>0.05$
Race[b]	White: 346 (80.0%) African American: 40 (9.2%) Asian: 7 (1.6%) Other or N/A: 40 (9.2%)	White: 231 (81.9%) African American: 27 (9.6%) Asian: 3 (1.1%) Other or N/A: 21 (7.4%) $\chi^2=1.6$ $p>0.05$
Household income in 1.000$[b]	Less than 25: 16 (3.7%) 25 to 50: 102 (24.1%) 50-75: 102 (24.1%) 75-100: 102 (24.1%) Over 100: 102 (24.1%)	Less than 25: 8 (2.8%) 25 to 50: 65 (23.0%) 50-75: 69 (24.4%) 75-100: 71 (25.1%) Over 100: 69 (24.4%) $\chi^2=0.9$ $p>0.05$
Mean testosterone level (ng/dl)[a]	Range: 4.5 to 574.4 Mean: 54.3 +/- 56.4	Range: 5.7 to 574.4 Mean: 60.7 +/- 59.1 $p=0.02$

[a] One sample t-test comparing the restricted sample to the average of the complete NIH cohort, which is deemed to be representative of the population

[b] χ^2 for goodness of fit using the complete NIH cohort proportions as the predicted model

Table 2: Demographic comparisons of males (n=127) versus females (n=155) subjects

	Males **n=127**	**Females** **n=155**
Age in years[a]	Range : 4.8 to 22.1 Mean : 13.1 +/-3.7	Range : 4.9 to 22.3 Mean : 13.1 +/-3.6 p=0.99
Handedness[b]	Right: 110 (86.6%) Left: 17 (13.4%)	Right: 142 (91.6%) Left: 13 (8.4%) χ^2=1.8 p=0.18
Race[b]	White: 108 (85.0%) African American: 11 (8.7%) Asian: 0 (0%) Other or N/A: 8 (6.3%)	White: 123 (79.3%) African American: 16 (10.3%) Asian: 3 (1.9%) Other or N/A: 13 (8.4%) χ^2=2.9 P=0.40
Household Income in 1.000$[b]	Less than 25: 1 (0.8%) 25 to 50: 33 (26.0%) 50-75: 35 (27.3%) 75-100: 31 (24.2%) Over 100: 28 (22.0%)	Less than 25: 7 (4.5%) 25 to 50: 32 (20.6%) 50-75: 34 (22.1%) 75-100: 40 (26.0%) Over 100: 41 (26.4%) χ^2=5.8 p=0.22
Mean testosterone level (ng/dl)[a]	Range: 5.7 to 535.6 Mean: 71.6 +/- 65.4	Range: 7.4 to 574.4 Mean: 52.7 +/- 52.8 p=0.001

[a] t test for independent samples
[b] χ^2 for independent samples

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