The Impact of Sex, Puberty, and Hormones on White Matter Microstructure in Adolescents

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Background: During adolescence, numerous factors influence the organization of the brain. It is unclear what influence sex and puberty have on white matter microstructure, as well as the role that rapidly increasing sex steroids play. Methods: White matter microstructure was examined in 77 adolescents (ages 10–16) using diffusion tensor imaging. Multiple regression analyses were performed to examine the relationships between fractional anisotropy (FA) and mean diffusivity (MD) and sex, puberty, and their interaction, controlling for age. Follow-up analyses determined if sex steroids predicted microstructural characteristics in sexually dimorphic and pubertal-related white matter regions, as well as in whole brain. Results: Boys had higher FA in white matter carrying corticospinal, long-range association, and cortico-subcortical fibers, and lower MD in frontal and parietal white matter compared with girls. Pubertal development was related to higher FA in the insula, while a significant sex-by-puberty interaction was seen in superior frontal white matter. In boys, testosterone predicted white matter integrity in sexually dimorphic regions as well as whole brain FA, whereas estradiol showed a negative relationship with FA in girls. Conclusions: Sex differences and puberty uniquely relate to white matter microstructure in adolescents, which can partially be explained by sex steroids.

Keywords: adolescence, diffusion tensor imaging, estradiol, puberty, testosterone

Introduction

Adolescence is the transition between childhood and adulthood, during which significant physical, social, and behavioral changes occur (Sisk and Foster 2004; Sisk and Zehr 2005). Most notably, during this time, the brain undergoes rapid development, including a reduction in gray matter volume (Giedd et al. 1999; Sowell et al. 2003) and an increase in white matter volume (Giedd et al. 1999), which coincides with maturation of cognitive processes and behavior. While these changes in brain organization and structure have largely been attributed to age, it is likely that other processes of development, such as puberty, contribute. Typically, beginning at age 10 in girls and age 12 in boys (McAnarney et al. 1992), puberty results in substantial increases in the primary sex steroids estradiol and testosterone. These steroids promote the maturation of secondary sex characteristics and ultimately the capability for reproduction. Animal studies suggest that these hormones also exert effects on brain maturation (Sisk and Foster 2004). While a number of studies have begun to examine the influence of puberty and sex on gray matter development (Giedd et al. 1999; Neufang et al. 2009; Peper et al. 2009; Bramen et al. 2011), the unique effects of puberty and sex steroids on white matter maturation, above and beyond age-related brain growth, remain largely unexplored. Furthermore, it remains unclear if testosterone and estradiol have similar or distinct effects on white matter microstructure in girls versus boys during adolescence.

Neuroimaging studies have provided great insight into a number of sex-specific differences in white matter development, suggesting possible pubertal influences on human brain structure. For example, during adolescence, boys show a steeper slope of increase in global white matter volume with age than girls, resulting in larger white matter volumes in boys compared with girls (Giedd et al. 1999; De Bellis et al. 2001; Lenroot et al. 2007). In addition to evidence examining gross brain morphology, sex differences are also apparent in white matter microstructure using diffusion tensor imaging (DTI). DTI is a noninvasive imaging technique that measures water diffusion displacement in order to make inferences about white matter fiber microstructure (Basser 1995). Two primary outcome variables of DTI include mean diffusivity (MD), a measure of overall water diffusion, and fractional anisotropy (FA), which reflects the degree of restricted, or anisotropic, diffusion. Higher FA values and lower MD values are believed to represent developmental processes of increased axon caliber, myelination, and/or fiber organization in white matter pathways (Beaulieu 2002; Alexander et al. 2007). Using DTI, Schmidt et al. (2008) found that in individuals 5–18 years of age, boys had higher FA in frontal, parietal, and occipito-parietal white matter compared with girls, whereas girls had higher MD values in the occipital–parietal regions. However, this study did not directly measure pubertal maturation to determine if puberty accounted for these differences or if pubertal status uniquely related to white matter microstructure, independent of sex. Also, the authors only reported FA and MD indices of diffusion for each significant white matter region, which is not a complete description of water diffusion. Axial diffusion or the amount of water diffusion along the primary axis or b vector (\(\lambda_1\)) and radial diffusion or the dispersion perpendicular to the primary axis of diffusion \([\lambda_2^{0.5}\lambda_3]/2\) can be examined within each FA and MD cluster to more fully characterize water diffusion. Axial and radial diffusivity values have also been associated with different biological underpinnings, and thus, understanding the relative contributions of these parameters to FA and MD values allows for a more thorough interpretation of potential sex differences between boys and girls. Nonetheless, these findings suggest that sex differences exist in white matter microstructure during development, which could be due to pubertal processes.

More recent evidence suggests that puberty may directly contribute to these sex differences in white matter maturation. Across 12 and 18 years of age, boys were shown to have larger...
reductions in corticospinal white matter density, which directly correlated with pubertal maturation; however, girls did not show this pattern (Perrin et al. 2009). Importantly, puberty-associated changes in white matter volume and density have been shown to be comparable to that of age (Perrin et al. 2009), suggesting that pubertal development may have an important role in gross structural brain organization and maturation during adolescence. At the microstructural level, only a few studies have examined differences in adolescent white matter microstructure between sexes, as well as in relation to puberty. Using magnetization transfer ratio (MTR), which assesses macromolecular content and structural integrity to estimate differences in myelination (McGowan 1999), Perrin et al. (2009) demonstrated that compared with girls, boys aged 12–18 showed a significant decrease in MTR values, reflecting less myelination, in parietal and occipital lobules. Furthermore, puberty was shown to significantly relate to these decreases in MTR values in boys but not in female adolescents, suggesting that typical developmental microstructural processes may follow both a pubertal-dependent and sexually dimorphic trajectory. More recently, Asato et al. (2010) also examined white matter microstructural differences in relation to puberty and sex in children, adolescents, and adults using DTI. Results showed white matter maturation to occur earlier in girls, which paralleled changes in pubertal status. However, this study examined the relationship of sex, puberty, and white matter microstructure only in brain regions that showed a positive relationship with age. Therefore, it is unclear if sex, puberty, or both may have unique and independent influences on white matter development in regions unrelated to age. Furthermore, the aforementioned studies examining these relationships included wide age ranges, and Asato et al. (2010) did not examine both radial and axial diffusion characteristics in order to fully characterize white matter microstructure. Thus, a more circumscribed analysis of how sex and puberty relate to white matter microstructure development during adolescence is warranted.

Beyond examining the influences of puberty and sex on white matter integrity in adolescents, it is important to determine if the rapid rise in gonadal steroids during this time contributes to microstructural brain organization and maturation during adolescence. Evidence suggests that some of the pubertal and sex relationships previously reported are likely due to the influence of sex steroids on white matter development in human adolescents. To our knowledge, only 3 studies have been published on this topic. Luteinizing hormone (LH) is involved in the activation of the hypothalamic-pituitary-gonadal axis during puberty. Higher concentrations of LH in prepubertal individuals (age 9) were positively associated with greater global white matter volume and regional white matter density (Peper et al. 2008). In addition, Perrin et al. (2008) examined the relationship between testosterone and white matter volume, as well as its composition using MTR in adolescents (ages 12–18). Testosterone positively related to white matter volume, while showing a negative relationship with MTR values, suggesting testosterone may relate to increases in axonal caliber. Furthermore, boys with a shorter androgen receptor (AR) gene polymorphism, leading to increased AR transcription levels, were shown to have a stronger positive relationship between testosterone and white matter volume compared with boys with a less active longer AR gene polymorphism (Perrin et al. 2008; Paus et al. 2010). However, these previous studies examined these associations over global lobar white matter, and thus, specificity of this relationship remains unclear. Furthermore, no studies have examined the relationship between estrogen and white matter microstructure in adolescents.

Thus, the goal of this study was to examine the relationships between white matter microstructure (FA and MD) and puberty, sex, and the primary sex steroids (estradiol and testosterone) in girls and boys, 10–16 years of age. Specifically, we first determined relationships between puberty, sex, and their interaction and white matter microstructure. Then, we employed region of interest (ROI)-based analyses to investigate the role of circulating levels of estradiol and testosterone on these regional sex differences in white matter microstructure, as well as those related to puberty. Lastly, exploratory whole-brain voxelwise analyses were performed to determine if sex hormones relate to white matter microstructure beyond these distinct sex and puberty-related regions. Following all FA and MD analyses, we also examined radial and axial diffusion to more fully characterize water diffusion in significant clusters. Importantly, using DTI, it has been shown that white matter microstructure develops with age and across adolescence (Schmithorst et al. 2002; Barnea-Goraly et al. 2005; Bava et al. 2010). In addition, estradiol and testosterone levels rise with age across adolescence (McAnarney et al. 1992). Therefore, in the current study, boys and girls were matched on age, and age was also statistically controlled for, allowing us to examine the “unique” relationships between white matter integrity, sex, and puberty, as well as between white matter integrity and sex hormones. Furthermore, pubertal hormones are produced in a sex-specific manner, so the influence of sex steroids on white matter microstructure was assessed in girls and boys separately.

Based on previous research showing greater changes in white matter volume, density, and microstructure in boys than girls (Giedd et al. 1999; Perrin et al. 2008, 2009), we hypothesized that boys would show higher values of FA and lower values of MD, indicative of greater axonal organization, axonal caliber, or myelination, than girls. In addition, we hypothesized that puberty would positively relate to white matter microstructural changes, including higher FA values and lower MD values, and that these relationships may be different between male and female adolescents. In addition, based on animal research, we hypothesized that testosterone would positively relate to white matter microstructure integrity in those regions in which boys show higher FA and lower MD than girls. In girls, we hypothesized that estradiol may also relate to higher values of FA and lower values of MD, but these relationships would be restricted to regions that have been shown to be larger in girls and have a large density of estrogen receptors, such as white matter tracts associated with the hippocampal and parahippocampal regions (McEwen 2001; Neufang et al. 2009).

**Materials and Methods**

**Participants**

Participants included 38 boys and 39 girls, ages 10–16 years. The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants were recruited and underwent comprehensive structured telephone interviews as part of an ongoing study focused on typical adolescent neurodevelopment. Written consent and assent was obtained from all
Hormonal Assessment

Four milliliters of blood was collected via venipuncture between the hours of 7:00 to 10:00 AM at the Oregon Clinical and Translational Research Institute in the same week as the imaging session. To reduce heterogeneity in female’s phase in menstrual cycle, laboratory visits for post-menarche girls occurred during the follicular phase (days 1 to 10) of their menstrual cycle. Total testosterone levels were determined by Coat-A-Count radioimmunoassay (Diagnostic Product Corp., Los Angeles, CA). The intraassay and interassay coefficients of variation (CVs) were 7.0% and 7.4%, respectively, at >10 ng/dL (lower level detection). Total estradiol levels were determined using the DSL-4800 Ultra-sensitive Estradiol Radioimmunoassay Kit (Beckman Coulter [formerly known as DSL], Fullerton, CA). The intraassay and interassay CVs were 7.4% and 12.6%, respectively, at >22 pg/mL (lower level detection). Data from 6 boys and 13 girls were excluded due to testosterone levels too low for reliable detection. For estradiol, 1 boy had levels too low for reliable detection and data for 1 girl was excluded due to an assay error. Thus, the final sample size for sex steroid analyses included 30 boys and 25 girls.

Participant Characteristics

Youth were administered the 2-subtest version of the Wechsler Abbreviated Scale of Intelligence (Wechsler 1999) to provide an estimate of overall intellectual functioning. Information was gathered on socioeconomic status (SES) using the Hollingshead Index of Socioeconomic Position (ISP) to parents, as part of the structured telephone interview. The Hollingshead ISP determines SES based on occupation and educational attainment of each parent (Hollingshead 1975). Pubertal maturation was assessed using the self-rating Pubertal Development Scale (PDS) (Petersen et al. 1988). The PDS consists of a total of 5 distinct questions for boys and girls. For boys, the 5 questions are in regards to height growth, body hair, skin changes, vocal changes, and facial hair. For girls, the 5 questions consist of assessing changes in height growth, body hair, skin, breast development, and menarche (both onset and duration of menstruation). For each of the 5 questions, youth were asked to rate their development on a 4 point scale (1 = does not begin yet, 2 = barely begun, 3 = definitely begun, 4 = seems complete), except for the menarche question for girls, which consisted of a yes/no answer choice. An average was then calculated from these responses for a pubertal development score ranging from 1 (prepubertal) to 4 (postpubertal). This questionnaire was chosen as it is a relatively noninvasive assessment of pubertal development and because self-reports on this scale have been shown to correlate significantly with other measures of pubertal status, including physician ratings (Petersen et al. 1988) and Tanner’s Sexual Maturation Scale self-ratings (Albertsson-Wikland et al. 1997).

Participant demographic information is presented in Table 1. IQ was not collected for one boy due to a technical error. Boys and girls did not significantly differ on age (t95 = 0.268, P = 0.79), SES (Hollingshead) (t95 = −2.32, P = 0.082), or IQ (t94 = −0.727, P = 0.478). Although groups were matched on age, girls matured earlier than boys, and thus as one would expect, significant group differences were seen in pubertal status (t95 = 3.20, P = 0.002), with girls being at a later stage of pubertal development than boys. Similarly, the subsample used for hormonal analyses also did not differ on age (t95 = 0.231, P = 0.81), SES (Hollingshead) (t95 = 0.161, P = 0.87), or IQ (t94 = 0.669, P = 0.51), but again significant group differences were seen in pubertal status (t95 = 3.40, P = 0.001). As expected, age and pubertal status were positively correlated for both boys (r90 = 0.69, P < 0.001) and girls (r95 = 0.74, P < 0.001).

Imaging Methods

Subjects were scanned on a 3.0-T Siemens Magnetom Tim Trio system (Siemens Medical Solutions, Erlangen, Germany) with a 12 channel head coil. Whole-brain structural anatomical images were acquired in the sagittal plane using a T1-weighted magnetization prepared rapid gradient echo scanning sequence (time to inversion = 900 ms, flip angle = 10°, time echo [TE] = 3.58 ms, time repetition [TR] = 2300 ms, acquisition matrix = 256 × 256, slice thickness = 1.1 mm). Diffusion weighted images (DWIs) were acquired oblique to the anterior commissure-posterior commissure, using a high-angular resolution echo planar imaging (EPI) sequence (TR = 9500 ms, TE = 95 ms, field of view = 240 mm2, 72 slices, slice thickness = 2 mm). Gradient encoding pulses were applied in 20 directions with a b-value of 1000 s/mm2, 4 diffusion-weighted runs were collected with 3 b0 (nodiffusion weighted) images per run. This diffusion-weighted protocol was used because previous research has shown that 20 diffusion gradient directions allow for calculating robust and reliable FA measurements (Li et al. 2005; Ni et al. 2006).

Image Processing and Analysis

Details of the DTI protocol and analyses have been previously reported (Herting et al. 2010, 2011). Briefly, eddy current effects, magnetic field in homogeneities, and head motion were corrected using FMRIB’s Diffusion Toolbox and Utility for Geometrically Unwarping EPI (Jenkinson 2003). The 4 DWI runs were aligned using linear (affine) registration to one another using FMRIB’s Linear Image Registration Tool (Jenkinson et al. 2002), averaged, and brain extracted using BET (Smith 2002). Based on the 6 motion parameters established by the affine registration of the b0s from each of the 4 runs, an average root mean square (RMS) was determined. Twelve participants (7 boys and 5 girls) had an excessive amount of movement, as defined as greater than 2 mm RMS for >5 run registrations. These individuals were excluded from all subsequent analyses (and were not reported in the demographics section). Analysis of Functional NeuroImages (Cox 1996) was then used to calculate the diffusion tensor and identify the eigenvalues of the tensor (λ1, λ2, λ3) for each voxel. FA and MD were determined for each voxel using AFNI’s nonlinear computational algorithm (3dDWItoDT; [Cox 1996]).
Voxelwise statistical analyses were performed on FA maps using tract-based spatial statistics (TBSS) (see Smith et al. 2006, 2007). First, a common registration target image was identified out of all individual subjects’ FA maps and affine aligned to standard MN1152 space. Second, each subject’s FA map was nonlinearly registered to this common target using FMRIB’s Non-linear Image Registration Tool (Andersson et al. 2007), and aligned FA images were averaged to create a group-wise mean FA map. Third, a white matter skeleton, representing only the major tracts common across all subjects, was created (for more detail, see Smith et al. 2006, 2007). A mean FA threshold of 0.2 was applied to the white matter skeleton to reduce partial volume effects (Smith et al. 2006). Fourth, each subject’s aligned FA image was projected onto the white matter skeleton for subsequent voxelwise group level statistics. To examine MD, MD images were also affine aligned to standardized space, the nonlinear registration parameters determined by FNIRT were applied to the MD maps, and MD images were merged and projected onto the FA-derived white matter skeleton to perform statistical comparisons. Due to the sensitivity of TBSS to outliers, FA and MD values for each cluster were examined for potential outliers >2.5 standard deviations (SDs) from the mean.

For a more comprehensive analysis of white matter microstructure, axial and radial diffusion were assessed in those clusters of FA and MD that were significantly related to sex, puberty, and sex-by-puberty interactions. In particular, FA and MD clusters were projected onto each subject’s axial and radial maps, and a mean radial and axial value was calculated for each cluster. Although complex in interpretation, particularly during adolescence (for review, see Paus 2010), these measures are 2 additional indices of water diffusion, with changes in radial diffusion (\(k\sqrt{\lambda_2 - \lambda_1}/2\)) often being useful to assess myelin in white matter, while changes in axial diffusion (\(\lambda_1\)) are thought to reflect axonal composition and coherence (Alexander et al. 2007).

**Sex and Puberty Analyses**

To examine the unique relationship between white matter microstructure and sex, puberty, and their interaction, voxelwise multiple regression analyses were performed on the FA and MD maps, while controlling for age, using AFNI’s 3dRegAna. Specifically, age, sex, pubertal status (derived from the PDS), and a sex-by-pubertal status interaction term were entered into the model to predict FA and MD. Monte Carlo simulations (Cox 1996) were performed for each separate analysis to correct for multiple comparisons. Specifically, an individual voxel probability threshold of \(P < 0.01\) and a cluster size of 29 contiguous voxels was used to reduce type 1 error. White matter tracts were identified using the MRI Atlas of Human White Matter, second edition (Oishi et al. 2011). To better understand group differences between sex, puberty, and their interaction, mean FA/MD values, as well as axial (\(\lambda_1\)) and radial (\(\lambda_2 + \lambda_3/2\)) diffusion, were extracted from significant clusters for each participant, and values were exported into PASW (Version 18; PASW, Chicago, IL). A 2-within (radial vs. axial diffusion), 2-between (sex) mixed model repeated measures analysis of covariance in PASW was then used to examine axial and radial values between the sexes, while controlling for age, in each significant FA and MD cluster. Independent two-sample t-tests were then performed to determine significant differences between the sexes on radial and axial diffusion. A modified Bonferroni correction was employed to determine statistical significance within FA and MD clusters (\(P < 0.0029\) for the 17 FA clusters; \(P < 0.025\) for the 2 MD clusters). Similarly, axial and radial values were also examined in relation to puberty and the sex-by-pubertal status interaction via multiple regression analyses. Specifically, pubertal or sex-by-pubertal status interaction was entered into the model to predict axial and radial values in significant FA clusters, while controlling for age. Statistical significance was determined as \(P < 0.05\) for these 2 clusters.

**Hormonal Analyses**

Using DTI ROI and whole-brain voxelwise analyses (Smith et al. 2006, 2007), both global and local specificity of relationships between white matter microstructure and sex hormones were examined in adolescents. To examine how sex steroids, testosterone and estradiol, related to sexually dimorphic and pubertal status-related white matter regions of FA and MD, individual multiple regressions were performed separately for boys and girls by putting age, estradiol, and testosterone in the model as predictors for FA and MD in significant sex and puberty-related clusters. Importantly, both testosterone and estradiol are derived in a similar fashion from cholesterol in boys and girls (Simpson et al. 2002). Testosterone can also be converted to estrogen via aromatase in the brain (Simpson et al. 2002). Therefore, by putting both estradiol and testosterone in the model, it is possible to determine if testosterone, estradiol, or both are associated with white matter microstructural characteristics in each sex. Furthermore, to determine which of the 3 variables (age, testosterone, or estradiol) were the best predictors of FA/MD in each cluster, stepwise regressions were also performed for each significant cluster. Given the exploratory nature of these analyses, \(P < 0.05\) was considered significant.

Exploratory regression analyses were then performed to examine the unique relationship between white matter microstructure and sex steroids (either estradiol or testosterone) in whole brain for both males and females using AFNI’s 3dRegAna. Again, Monte Carlo simulations (Cox 1996) were performed to correct for multiple comparisons, and an individual voxel probability threshold of \(P < 0.01\) and a cluster size of 29 contiguous voxels was used to determine statistical significance. Similarly to puberty and sex analyses, for a more comprehensive analysis of white matter microstructure, axial and radial diffusion were also assessed in those clusters of FA and MD that were significantly related to pubertal hormones. Again, sex hormones were entered into the model to predict axial and radial diffusion in significant clusters, while controlling for age. Bonferroni correction was employed to determine statistical significance within FA and MD clusters (boys: testosterone and FA [6 clusters], \(P < 0.0085\); estradiol and FA [4 clusters], \(P < 0.0125\); testosterone and MD [1 cluster], \(P < 0.05\); girls: estradiol and FA [2 clusters], \(P < 0.025\); testosterone and FA [1 cluster], \(P < 0.05\)).

**Data Analyses**

All statistical analyses were carried out using PASW (18, PASW, Chicago, IL). Normality was verified using Kolmogorov-Smirnov tests, and transformations were used, as appropriate. Exploratory data analyses revealed that pubertal status and estradiol values were not normally distributed for either boys or girls. Therefore, a reflection square-root transformation \([(\text{sqrt}(K - M)) \times (-1))\] \(K = \text{constant}; M = \text{individual's PDS score}\) and a log transformation were applied, leading to a normal distribution of pubertal status and estradiol in boys and girls. Testosterone was also not normally distributed for girls, but a sin transformation was applied to obtain a normal distribution. Pearson’s r correlations were used to examine the relationships between age, pubertal status, estradiol, and testosterone in both boys and girls.

**Results**

**Sex Differences in White Matter FA and MD**

Voxelwise regression analyses predicting FA and MD, with sex, puberty, and sex-by-puberty interactions as predictors, while controlling for age, revealed sex differences in a number of white matter regions (Fig. 1a and Table 2). Boys showed higher FA in white matter compared with girls throughout the brain, including the superior cerebellar peduncle (SCP), posterior limb of the internal capsule (PLIC), middle/lateral occipital gyrus (O2 WM), superior temporal gyrus (STG WM), inferior frontal gyrus (IFG WM), inferior fronto-occipital (IFO) fasciculus, thalamus (TH), midbrain (M), cingulum (CG), and supramarginal gyrus (SMG WM). No regions were detected where girls had higher FA compared with boys. Sex differences in MD showed an opposite pattern than that of FA, with boys showing lower MD than girls in white matter of the superior temporal (STG WM) and superior frontal gyrus (SFG WM) (Fig. 1b and Table 2).
To further elucidate sex differences in white matter microstructure, radial and axial diffusivity was determined for each significant FA and MD cluster. In white matter regions where boys had higher FA values than girls, boys had overall lower radial or higher axial diffusivity values compared with girls, except in one cluster in the STG. However, after Bonferroni correction, significant radial diffusion differences between the groups were seen only in the SCP, PLIC, as well as portions of O2, IFO fasciculus, thalamus, STG, and midbrain; higher values of axial diffusivity in O2 and cingulum in boys remained significant compared with girls (Supplementary Table 1). Furthermore, in the 2 clusters showing sex difference in MD, girls had significantly higher axial diffusivity in the SFG white matter, as well as significantly higher axial and radial diffusivity in white matter in the STG compared with boys (Supplementary Table 1).

**Pubertal Status Relates to White Matter FA and MD**

After controlling for age, FA was found to positively relate to puberty in one white matter region (Fig. 2a and Table 3); the right insular gyrus (IG WM). There were no negative relationships found between puberty and FA. Furthermore, no relationships were seen between puberty and MD. Lastly, a significant sex-by-puberty interaction was found for FA in the

![Figure 1. Sex differences in FA and MD, after controlling for age. Coronal views of differences in FA and MD, represented by t-statistic maps overlaid on the mean FA skeleton (green) and a standardized brain. (a) Sex effect for FA: Blue-Light Blue = FA is greater in boys compared with girls; (b) Sex effect for MD: Pink = MD is less in boys compared with girls. Results have been dilated for viewing purposes. Abbreviations: L, left; R, right; CG, cingulum; IFG WM, inferior frontal gyrus white matter; IFO, inferior fronto-occipital fasciculus; M, midbrain; O2 WM, middle/lateral occipital gyrus white matter; PLIC, posterior limb of the internal capsule; SCP, superior cerebellar peduncle; SFG WM, superior frontal gyrus white matter; SMG WM, supramarginal gyrus white matter; STG WM, superior temporal gyrus white matter; TH, thalamus.](http://cercor.oxfordjournals.org/)

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SFG (Fig. 2b and Table 3), with boys showing a positive relationship and girls showing a negative relationship between FA and pubertal development. No significant sex-by-puberty interactions were seen for MD. Follow-up assessment of radial and axial diffusion in these clusters of FA revealed that radial diffusivity in IG white matter significantly decreased with puberty, whereas no changes in axial diffusion were seen (Supplementary Table 2). In addition, axial diffusion increased with pubertal development in boys, but radial diffusion increased with puberty in girls in the SFG (Supplementary Table 2).

Hormonal Assessment

The mean and SDs, as well as the range of total estrogen and testosterone values for both sexes can be seen in Table 1. Although estrogen and testosterone values were transformed to obtain normality, untransformed values are reported in the table. As expected, in boys, testosterone positively related to age ($r_{50} = 0.52, P < 0.003$) and pubertal status ($r_{50} = 0.72, P < 0.001$), whereas these relationships were not seen with estradiol (age: $r_{50} = 0.212, P = 0.26$; pubertal status: $r_{50} = 0.081, P = 0.67$). In girls, however, estradiol did not relate to age ($r_{25} = -0.161, P = 0.44$) or pubertal status ($r_{25} = 0.025, P = 0.91$), and neither did testosterone (age: $r_{25} = 0.062, P = 0.77$; pubertal status: $r_{25} = -0.061, P = 0.77$). The nonsignificant relationship between age and estradiol was likely due to collection of hormone data during the follicular phase of the menstrual cycle, where both pre-menarche and post-menarche estradiol levels are low (McAnarney et al. 1992).

Sex Hormones Predict FA in Sexually Dimorphic Regions

Multiple regression analyses showed higher testosterone levels significantly predicted higher FA values in the PLIC, IFG, thalamus, and midbrain in boys (Table 4). Similar trends were seen for additional clusters in the PLIC, thalamus, SFG, IFG, and STG white matter. In addition, estradiol also positively predicted increased FA in both thalamic white matter clusters, and a trend was also seen between estradiol and FA in 1 of the 2 O2 white matter clusters. While age did not significantly predict FA in any of these clusters (only trend-level relationships were seen between FA and MD, and the STG, and SFG), age negatively predicted MD in both the STG and SFG. Stepwise regression analyses supported the previous findings, showing that testosterone was the best predictor of FA in 10 of the 17 FA clusters (Table 4), including the PLIC, STG, IFG, thalamus, midbrain, and the IFO fasciculus. Estradiol was also found to be the best predictor for FA in one of the O2 clusters. In addition, stepwise regression revealed that age was the best predictor of MD in both the STG and SFG, with decreases in MD seen with age in these regions.

In contrast to boys, estradiol largely did not relate to FA values in the sexually dimorphic clusters, except for 2 clusters in which a significant negative relationship was seen between FA and estradiol in the PLIC (Table 5). Instead, age seemed to be the better predictor of FA in a number of these clusters, including significant positive relationships seen between the midbrain and occipital white matter, and positive trends seen in PLIC and SMG white matter. No relationships were seen between MD and age, testosterone, or estradiol for the 2 MD clusters. Again, stepwise regression analyses revealed that estradiol was the best predictor for FA in 2 of the clusters in the PLIC, with higher estradiol values relating to decreases in FA, while age was the best predictor for FA in midbrain clusters and O2 white matter (Table 5).

No significant relationships between sex steroids and FA in the IG (pubertal effect cluster) or the SFG white matter (sex-by-pubertal interaction cluster) were seen for boys or girls, although a positive trend was seen between testosterone and FA in the SFG. A significant positive relationship, however, was seen between FA in IG white matter and age in girls, and stepwise regression analyses confirmed that age was, in fact, the best predictor of FA in this region.

Table 2

<table>
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<th>White matter tracts</th>
<th>Number of voxels</th>
<th>MNI coordinates $x$ $y$ $z$</th>
<th>Mean value (SD)</th>
<th>$P$ value</th>
<th>Effect size</th>
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<td></td>
<td><strong>Boys</strong></td>
<td><strong>Girls</strong></td>
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<td>0.67 (0.04)</td>
<td>0.64 (0.04)</td>
<td>0.001</td>
</tr>
<tr>
<td>L. STG WM</td>
<td>63</td>
<td>$-54$ $-14$ $1$</td>
<td>0.34 (0.05)</td>
<td>0.31 (0.05)</td>
<td>0.011</td>
</tr>
<tr>
<td>R. IGF WM</td>
<td>57</td>
<td>$41$ $40$ $-5$</td>
<td>0.39 (0.04)</td>
<td>0.35 (0.05)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>R. IFO fasciculus</td>
<td>53</td>
<td>$38$ $-43$ $-13$</td>
<td>0.35 (0.05)</td>
<td>0.30 (0.04)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>L. thalamus</td>
<td>45</td>
<td>$-17$ $-23$ $-3$</td>
<td>0.48 (0.03)</td>
<td>0.44 (0.04)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>R. midbrain</td>
<td>41</td>
<td>$3$ $-16$ $-10$</td>
<td>0.33 (0.03)</td>
<td>0.30 (0.03)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>R. cingulum</td>
<td>38</td>
<td>$-9$ $-18$ $31$</td>
<td>0.58 (0.05)</td>
<td>0.54 (0.04)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>R. O2 WM</td>
<td>37</td>
<td>$22$ $-86$ $2$</td>
<td>0.39 (0.06)</td>
<td>0.34 (0.06)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>R. SMG</td>
<td>37</td>
<td>$54$ $-33$ $19$</td>
<td>0.35 (0.07)</td>
<td>0.29 (0.08)</td>
<td>0.001</td>
</tr>
<tr>
<td>R. midbrain</td>
<td>32</td>
<td>$10$ $-24$ $-12$</td>
<td>0.46 (0.03)</td>
<td>0.42 (0.04)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>L. thalamus</td>
<td>31</td>
<td>$-14$ $-23$ $2$</td>
<td>0.33 (0.03)</td>
<td>0.30 (0.03)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>R. IFO fasciculus</td>
<td>30</td>
<td>$-26$ $15$ $-9$</td>
<td>0.46 (0.05)</td>
<td>0.42 (0.06)</td>
<td>0.001</td>
</tr>
<tr>
<td>L. STG WM</td>
<td>30</td>
<td>$-50$ $-41$ $18$</td>
<td>0.40 (0.05)</td>
<td>0.36 (0.08)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>MD (µm²/ms): boys &lt; girls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. STG WM</td>
<td>30</td>
<td>$-56$ $-15$ $1$</td>
<td>0.65 (0.05)</td>
<td>0.70 (0.05)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>L. SFG WM</td>
<td>29</td>
<td>$-16$ $-13$ $58$</td>
<td>0.69 (0.02)</td>
<td>0.72 (0.04)</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

Note: Name, voxel number, and Montreal Neurological Institute (MNI) coordinates of center of mass for each cluster are given, as well as the mean and SD of FA and MD values for each cluster for boys and girls, respectively. $P$ value and effect size (Cohen’s $d$) are reported for the group difference for each cluster. L, left; R, right; WM, white matter.
Whole-Brain Relationships between Sex Hormones and Microstructure

Results for the voxelwise regression analysis examining sex hormones (either testosterone or estradiol) as a predictor of FA in boys, while controlling for age, can be seen in Figure 3 and Table 6. In boys, testosterone was related to higher FA values in a number of white matter regions, including the left STG, body of the corpus callosum (CC), SFG, PLIC, and the angular gyrus (AG WM). In addition, one negative relationship was seen between testosterone levels and FA in the middle cerebellar peduncle (MCP) (Fig. 3a). Testosterone also positively related to MD in SFG white matter in boys (Fig. 3c and Table 6). Similar to testosterone, estradiol was also related to higher FA values in the bilateral inferior cingulum (near the hippocampus), SFG, and precuneus (PCun WM) white matter (Fig. 3b and Table 6). No significant relationships were found between estradiol and MD in boys. In girls, voxelwise regression analyses examining estradiol as a predictor of FA, after controlling for age, revealed that estradiol was negatively associated with FA in the right AG white matter and the superior longitudinal fasciculus (SLF) (Fig. 3d and Table 6). Similar to boys, testosterone also showed a positive relationship with FA in girls, although this relationship was only seen in a small region of precentral gyrus white matter (PrG WM) (Fig. 3e and Table 6). No significant relationships were found between either estradiol or testosterone and MD in girls. To ensure that the above relationships were sex hormone specific to testosterone or estradiol and not a combination of both, analyses were also performed controlling for testosterone when examining estradiol and vice versa. In all analyses, the other sex hormone did not significantly predict FA or MD values in these clusters ($P$’s greater than $>0.1$), and all results remained significant ($P$’s $<0.002$), suggesting that these results are hormone specific.

To further characterize how sex hormones relate to white matter microstructure, radial and axial diffusivity were determined for each significant FA and MD cluster. These results can be seen in Supplementary Table 3. Overall, lower radial, higher axial diffusivity values, or both were seen for white matter regions where testosterone and estradiol levels related to higher FA in boys. The opposite pattern was seen in clusters where estradiol levels related to lower levels of FA in girls, with higher radial and lower axial diffusivity relating to hormone levels in these clusters (Supplementary Table 3).

Discussion

The current study examined the unique relationships between white matter microstructure, sex, puberty, and sex hormones in a large sample of youth ages 10–16, while controlling for age. Consistent with our hypotheses, boys had significantly higher FA values than girls in a number of white matter regions, many of which are known to carry fibers of the corticospinal tract, as well as some long-range white matter tracts and regions that carry limbic white matter fibers. Furthermore, girls had higher values of MD than boys in the white matter of the SFG and STG.
After controlling for age, puberty was only seen to positively relate to white matter integrity in the IG, and only a single significant sex-by-pubertal interaction was seen, located in the SFG. Moreover, in boys, testosterone predicted higher FA in these sexual dimorphic regions, while estradiol seemed to have little relationship to sex-related differences in girls. Most surprisingly, exploratory analyses, again controlling for age, showed that, in boys, testosterone and estradiol predicted higher FA in a number of additional fiber tracts that were not related to sex or pubertal development in boys. However, in girls, testosterone showed a less robust relationship with FA, while estradiol actually showed a negative relationship with FA in the AG and SLF.

Our findings are in agreement with previous studies, but here we confirm that robust sex differences exist in FA and MD in adolescents, with boys having higher FA and lower MD...
compared with girls, while controlling for age and puberty. These sex differences in FA were widespread and seen in regions known to carry long-range association fibers (IFO fasciculus), as well as cortico spinal (SCP, midbrain) and limbic fiber tracts (cingulum, PLIC, thalamus). In addition, boys also showed higher FA and lower MD values in cortical white matter areas (IFG WM, SFG WM, STG WM, SMG WM, O2 WM). These findings are consistent with previous literature on sex
differences in the adolescent brain (for review, see Lenroot and Giedd 2010), including adolescent boys showing higher FA and lower MD in bilateral frontal white matter regions (Silveri et al. 2006; Schmithorst et al. 2008), as well as the left parietal and parieto-occipital white matter compared with girls (Schmithorst et al. 2008). However, prior studies only reported differences in FA and MD, which if presented alone, do not fully characterize differences in diffusion between the sexes. Here, we also examined the changes in radial and axial diffusion within significant FA and MD clusters, which allows for a more clear understanding of these sex-related differences that has been largely absent in the previous literature. These findings clearly show that sex differences in FA were largely driven by boys having less radial diffusion and greater axial diffusion, whereas girls had higher axial diffusivity in the MD clusters. In mice, higher values of FA and lower values of radial diffusivity have been thought to reflect greater myelination (Song et al. 2005), suggesting one possible interpretation of these findings is that boys may have greater myelination in these regions compared with girls. However, DTI indices are not selective markers of specific neurobiological properties, and other tissue properties, such as greater axonal directional coherence, a reduction in the number of crossing fibers, and axonal density, may contribute to decreased radial diffusivity in boys versus girls. During human adolescence especially, it cannot be ruled out that differences in FA and radial diffusivity reflect both differences in myelination, the ratio between myelin and axon caliber, or both, between boys and girls (for review, see Paus 2010).

Despite the fact that the exact neurobiological processes underlying these differences are unable to be determined, widespread sex differences in FA were apparent, and a number of them may help to explain distinct gender-based behavioral differences. For example, sex differences in the corticospinal fibers may contribute to differences in motor learning and motor skill proficiency seen between the sexes, as boys have been shown to typically out-perform age-matched girls (Dorfberger et al. 2009; Barnett et al. 2010). Additionally, the higher values of FA in boys compared with girls in limbic-associated white matter tracts may relate to sex differences in emotional processing (for review, see Hamann and Canli 2004). For example, in a meta-analysis of 65 studies, women were shown to have greater peak activity in the anterior cingulate and thalamus in response to emotionally valenced stimuli, whereas men showed greater peak activity in the IFG and posterior parietal and occipital cortices (Wager et al. 2003). Because the prefrontal cortex interacts with the limbic system to assist in the processing and regulation of emotion (Quirk and Beer 2006), having lower FA in limbic regions may reflect less structural connectivity between cognitive and emotional brain regions in girls. This could possibly contribute to these previously reported increases in limbic brain activity to emotional stimuli in women, as well as more overt expression of emotions reported in women (Kring and Gordon 1998). In this regard, differences in white matter microstructure in girls versus boys may not only contribute to variations in emotional perception, but may also help to explain the increased prevalence for mood disorders among female adolescents (Angold et al. 1999). Therefore, more research is warranted to determine how the current findings relate to a number of behavioral differences seen in male and female adolescents.

Unlike the robust number of sex differences seen in the current study, fewer relationships between puberty and white matter microstructure were observed. In both sexes, puberty was related to higher FA, driven by decreases in radial diffusivity, in IG white matter. The IG is involved in emotion

### Table 6
Significant relationships between sex hormones and whole-brain FA and MD in boys and girls, after controlling for age

<table>
<thead>
<tr>
<th>White matter tracts</th>
<th>Number of voxels</th>
<th>MNI coordinates</th>
<th>Direction of relationship</th>
<th>( \beta )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( x )</td>
<td>( y )</td>
<td>( z )</td>
<td></td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone and FA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. STG WM</td>
<td>92</td>
<td>−53</td>
<td>−36</td>
<td>12</td>
<td>Positive</td>
</tr>
<tr>
<td>L. CC (body)</td>
<td>59</td>
<td>−11</td>
<td>14</td>
<td>23</td>
<td>Positive</td>
</tr>
<tr>
<td>L. SFG WM</td>
<td>56</td>
<td>−18</td>
<td>37</td>
<td>21</td>
<td>Positive</td>
</tr>
<tr>
<td>R. PLIC</td>
<td>42</td>
<td>20</td>
<td>−1</td>
<td>17</td>
<td>Positive</td>
</tr>
<tr>
<td>L. MCP</td>
<td>33</td>
<td>−16</td>
<td>−43</td>
<td>−34</td>
<td>Negative</td>
</tr>
<tr>
<td>R. AG WM</td>
<td>32</td>
<td>44</td>
<td>−42</td>
<td>39</td>
<td>Positive</td>
</tr>
<tr>
<td>Estradiol and FA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. cingulum (hippocampal part)</td>
<td>54</td>
<td>−22</td>
<td>−36</td>
<td>−7</td>
<td>Positive</td>
</tr>
<tr>
<td>R. cingulum (hippocampal part)</td>
<td>50</td>
<td>22</td>
<td>−34</td>
<td>−8</td>
<td>Positive</td>
</tr>
<tr>
<td>L. SFG WM</td>
<td>44</td>
<td>11</td>
<td>3</td>
<td>61</td>
<td>Positive</td>
</tr>
<tr>
<td>R. PCun WM</td>
<td>33</td>
<td>11</td>
<td>−63</td>
<td>35</td>
<td>Positive</td>
</tr>
<tr>
<td>Testosterone and MD (( \mu \text{m}^2/\text{ms} ))</td>
<td>32</td>
<td>−12</td>
<td>57</td>
<td>−9</td>
<td>Positive</td>
</tr>
<tr>
<td>Estradiol and MD (( \mu \text{m}^2/\text{ms} ))</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Girls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol and FA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. AG WM</td>
<td>96</td>
<td>30</td>
<td>−59</td>
<td>38</td>
<td>Negative</td>
</tr>
<tr>
<td>L. SLF</td>
<td>37</td>
<td>−30</td>
<td>−40</td>
<td>34</td>
<td>Negative</td>
</tr>
<tr>
<td>Testosterone and FA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. PG WM</td>
<td>42</td>
<td>36</td>
<td>3</td>
<td>20</td>
<td>Positive</td>
</tr>
<tr>
<td>Estradiol and MD (( \mu \text{m}^2/\text{ms} ))</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Testosterone and MD (( \mu \text{m}^2/\text{ms} ))</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note: Name, voxel number, and Montreal Neurological Institute (MNI) coordinates of center of mass for each cluster are given. Direction of relationship, \( \beta \) and \( P \) value are also reported for each cluster. L, left; R, right; WM, white matter.
and cognition, and this relationship may be related to changes in emotional processing that occur between childhood and adulthood (Choudhury et al. 2006). In addition, a significant sex-by-pubertal interaction was seen in the SFG, with boys showing greater FA with puberty, driven by increases in axial diffusivity, and girls showing less FA with advanced pubertal status, driven by increases in radial diffusivity. These findings are congruent with previous studies that have also shown puberty to relate to microstructural changes in a sex-specific manner. Specifically, Perrin and colleagues found that puberty was significantly related to decreases in MTR values (suggesting changes in axonal caliber) in boys but not female adolescents in the corticospinal tract and fornix (Perrin et al. 2009). However, we found pubertal development to relate to white matter microstructure in a sex-dependent manner only in the SFG. In fact, we did not find a relationship between puberty and white matter microstructure in regions known to carry corticospinal tract fibers, but rather found sex to be the best predictor of FA in these regions (PLIC, midbrain, SCP, thalamus). Furthermore, our results are in disagreement with those of Asato et al. (2010), which reported that girls, not boys, showed earlier maturation of white matter microstructure (decreases in radial diffusivity), and these changes paralleled pubertal development. The discrepancies between previous studies and the current findings may be due to differences in methodology, as MTR was summed across all 4 lobes of the brain in the former study (Perrin et al. 2009), and anisotropy relationships with sex and puberty were only examined in brain regions showing age-related changes in water diffusion in the latter study (Asato et al. 2010). In addition, pubertal development was used in the previous studies as a categorical variable during analyses and either collapsed across pubertal stages (Asato et al. 2010) or only included for subjects in mid- to late-pubertal development (Perrin et al. 2009). In the current study, however, we utilized a voxelwise analysis technique and used pubertal status as a continuous variable to examine the unique relationships between sex, puberty, and white matter microstructure. Thus, using the current methods, indentifying developmentally distinct relationships between sex or puberty and well-defined white matter tracts was feasible and may account for differences in results across studies.

Beyond the sex and pubertal relationships seen with white matter microstructure, the current paper investigated the influence of sex steroids on sex and pubertal-specific white matter microstructure. To our knowledge, this is the first study to examine how sex hormones relate to white matter microstructure in adolescents using DTI and the first to examine the relationship between estradiol and white matter microstructure in adolescents. Here, we showed that in boys, testosterone predicted white matter integrity in regions in which sex differences in white matter microstructure have been previously shown in adolescents, including regions that carry fibers from the corticospinal tract (SCP, PLIC, midbrain, thalamus), as well as in cortical white matter (STG WM, IFG WM) and long-association fibers (IFO fasciculus). These findings are largely in agreement with recent work showing a relationship between gross white matter volumes and testosterone in male, but not female, adolescents (Perrin et al. 2008) and that having a more efficient AR genotype (suggesting greater testosterone levels) is related to increases in global white matter volume in male adolescents (Perrin et al. 2008; Paus et al. 2010). Interestingly, follow-up whole-brain analyses in the current study showed that both testosterone and estradiol relate to greater FA in a number of brain regions in boys that were not specifically seen to be sex or puberty related. Together with the current results, these finding suggest that testosterone not only influences sex differences in gross volumetric indices but also microstructural characteristics of white matter during adolescence.

One of the most striking findings of the current study is that, unlike testosterone in boys, estradiol did not relate to as many regions of FA differences between the sexes in girls and actually showed a negative relationship with FA in both sexually dimorphic areas, as well as regions unrelated to sexual phenotype. These findings are contrary to our hypothesis that estradiol would relate to higher values of FA in hippocampal and parahippocampal white matter and are in opposition to other research suggesting that estradiol levels relate to volumetric differences in parahippocampal gray matter, which densely expresses estrogen receptors (McEwen 2001; Neufang et al. 2009). Rather, our findings are supported by other studies showing either no relationship or a negative relationship between estradiol and other brain structure in girls. For example, testosterone has been found to be positively associated with global gray matter density in boys, whereas estradiol shows a negative relationship with global and regional gray matter density in girls (Peper et al. 2009). Furthermore, these results are in agreement with animal research showing that estrogen decreases the rate of myelination during puberty, resulting in more myelinated fibers in the corpus callosum of male compared with female adolescent rats (Juraska and Markham 2004). Specifically, removal of the ovaries just prior to puberty in female rats results in an increase in the number of myelinated axons in the splenium of the corpus callosum, which can be readily reversed with chronic estrogen treatment (Juraska and Markham 2004). While it is unclear if this effect of estradiol is seen in other regions of the brain in females, it seems plausible that such a process may contribute to the sex differences seen in FA in the PLIC, and the negative relationships seen between estradiol and FA in the AG and the SLF in the current study. Not only did radial diffusivity largely account for the sex differences seen in this area, suggesting differences in myelination may be a contributing factor, but also a negative relationship was seen between estradiol and FA in these regions in girls. However, despite this relationship in girls, estradiol seems to have a strikingly different relationship with white matter microstructure in boys. This was highlighted by our current results showing a positive relationship between estradiol and FA in a few regions in boys, including bilateral cingulum, near the hippocampus. While unexpected, these findings further support the idea that sex hormones, both estradiol and testosterone, have a very different profile in regards to their relationship with white matter maturation between the sexes. A possible reason for this difference may be that oligodendrocytes—the brain cells essential for myelination—are more abundant in the adult male rat brain compared with the female brain, and proliferation of these cells is increased by sex steroid levels (Cerghe 2009). Furthermore, because estrogen receptors and ARs are found on both neurons, as well as oligodendrocytes (Finley and Kritzer 1999; Zhang et al. 2004), it is possible that one mechanism by which these hormones may influence sex-specific white matter microstructure during adolescence is by both testosterone and estradiol facilitating myelination and premyelination events in...
boys, while estradiol may suppress these effects in girls. Alternatively, other research has shown that continual exogenous estradiol and testosterone administered at birth and through adulthood actually produce the opposite effect, with estradiol increasing white matter maturation (Prayer 1997), suggesting that the increase in pubertal hormones may have distinct and varying effects on white matter development depending on both sex and the timing of the surge of pubertal hormone. Moving forward, it will be important to fully understand the mechanisms by which pubertal increases in sex hormones influence sex-specific white matter microstructure across adolescence. Specifically, animal studies comparing DTI results with postmortem analyses of white matter microstructure, and in relation to pubertal rises in sex steroids, are warranted to further elucidate the effects of sex steroids on brain organization and structure during adolescence.

Lastly, there were some clusters of sex difference in FA in which neither estradiol nor testosterone were predictive. Estradiol and testosterone also did not predict FA in IG (pubertal status effect) or SFG white matter (sex-by-pubertal interaction) or in the MD sex clusters in boys or girls. It remains unclear why testosterone and estradiol predict microstructural characteristics in some white matter regions but not others. However, these results likely speak to the fact that there are a number of intrinsic and extrinsic sex differences between boys and girls that may be independent of age and pubertal maturation. Similarly, the lack of relationship between sex steroids and FA in clusters in pubertal-related white matter clusters may be due to the fact that our definition of pubertal development (as defined by the Pubertal Developmental Scale) is not completely synonymous with sex steroid circulation. In other words, pubertal development encompasses a number of physiological and psychological changes that may or may not be solely due to changes in the level of testosterone and estradiol during this time. Therefore, additional physical and hormonal changes (e.g., growth hormones, prepubertal hormones) that occur with puberty may help to clarify pubertal influences on white matter microstructure.

While this is the first study of its kind, several limitations should be acknowledged. First, we did not find a positive relationship between age and estradiol in the current sample. This is likely due to the timing of blood samples during the follicular phase when estradiol levels are uniformly low (McAnarney et al. 1992). Although collecting estradiol levels during the follicular phase had a number of advantages (such as being able to readily confirm subjects’ phase, using menstruation onset as a guideline), it is unclear if more robust findings may have been observed if data were collected during the luteal phase, when estradiol levels may have shown greater variability. Because age was statistically controlled for during analyses, it is unlikely that a lack of relationship between estradiol and age in females would have contributed to the few relationships found between estradiol and white matter microstructure. However, it cannot be ruled out that more widespread relationships between estradiol and white matter microstructure may exist in girls, if data were collected during a different point in the menstrual cycle. Secondly, the results here are based on a cross-sectional design, and despite the fact that boys and girls were closely matched on a number of demographic variables, girls reported higher values of pubertal maturation. Although this is to be expected based on previous findings that girls mature on average of 1–2 years earlier than boys (McAnarney et al. 1992), it makes examining puberty and sex-specific development challenging. Similarly, since pubertal status and age are highly correlated, it is often difficult to partial out pubertal effects that are independent of age. In the current study, we chose to statistically control for this difference in puberty between the sexes and the potential confound of age in regards to pubertal-related changes by putting age, puberty, sex, and sex-by-pubertal interaction as independent predictors in the regression model for white matter microstructure. By doing so, we were able to examine the unique relationships between each predictor and white matter integrity. Nonetheless, because age and puberty are collinear, statistically controlling for age, although necessary, may have contributed to detecting only a small number of relationships between puberty and FA/MD. Replicating the current findings by utilizing a longitudinal study design is necessary to fully track the developmental trajectories of puberty in boys and girls. In addition, there are likely intrinsic and extrinsic differences between boys and girls prior to and during puberty, which cross-sectional studies are unable to account for. Thus, sex- and puberty-related neurodevelopment, independent of age, across adolescence is an important endeavor for future research.

In summary, our results show that widespread sex differences exist in white matter microstructure in adolescents ages 10–16. Follow-up analyses revealed testosterone and estradiol to have distinct relationships with white matter microstructure in these sexually dimorphic regions, as well as in the whole brain, with higher testosterone predicting greater FA in boys, while estradiol predicted lower FA values in girls. We conclude that sex differences and puberty uniquely relate to white matter microstructure in adolescents, and these relationships can be partially explained by sex steroids depending on sex and brain region. It will be important moving forward to determine if these sex and hormone relationships contribute to distinct cognitive profiles in adolescents, as well as if they differentially relate to sex-specific vulnerabilities for psychopathology.

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Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

Notes
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References


