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Sex Differences and the Impact of Steroid Hormones on the Developing Human Brain

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Little is known about the hormonal effects of puberty on the anatomy of the developing human brain. In a voxel-based morphometry study, sex-related differences in gray matter (GM) volume were examined in 46 subjects aged 8–15 years. Males had larger GM volumes in the left amygdala, whereas females had larger right striatal and bilateral hippocampal GM volumes than males. Sexually dimorphic areas were related to Tanner stages (TS) of pubertal development and to circulating level of steroid hormones in a subsample of 30 subjects. Regardless of sex, amygdala and hippocampal volumes varied as a function of TS and were associated with circulating testosterone (TEST) levels. By contrast, striatal GM volumes were unrelated to pubertal development and circulating steroid hormones. Whole-brain regression analyses revealed positive associations between circulating estrogen levels and parahippocampal GM volumes as well as between TEST levels and diencephalic brain structures. In addition, a negative association was found between circulating TEST and left parietal GM volumes. These data suggest that GM development in certain brain regions is associated with sexual maturation and that pubertal hormones might have organizational effects on the developing human brain.

Keywords: brain morphometry, sex differences, sexual maturation, steroid hormones

Introduction

Adolescence is an important developmental period in which major physical, psychological, cognitive, and social transformations occur (Blakemore and Choudhury 2006; Galvan et al. 2007; Markham et al. 2007) and gender differences emerge and manifest themselves (Lenroot et al. 2007; Schmithorst et al. 2007). Behavioral transformations are closely related to cerebral development, encompassing dramatic and widespread changes in brain morphology. Although basic developmental processes are comparable between boys and girls, such as linear increases in white matter (WM) volume and nonlinear inverted u-shaped development of regional gray matter (GM), sexual dimorphisms have been reported for global and regional brain volumes and the time course of brain development (Giedd et al. 1997, 1999). Whereas peaks in GM volume typically occur 1 year earlier in girls than in boys, the rates of global volume changes follow a steeper slope in boys than in girls with respect to both increase of WM and decrease in GM volumes. Likewise, volumes of subcortical structures like the striatum, the hippocampal, and the amygdala change during adolescence in a sex-dependent manner, with amygdala volume increasing significantly more in males than in females and hippocampal and striatal volume increasing more in females (Caviness et al. 1996; Giedd et al. 1997; Lange et al. 1997).

Although it has recently been demonstrated that sexual dimorphism in total cerebral and subcortical GM and WM volumes are already present in the neonatal brain (Gilmore et al. 2007), many sex differences in brain structures seem to occur after the age of 9 or 10 (see, e.g., Goldstein et al. 1999; Giedd et al. 2006). Recently, De Bellis et al (2001) demonstrated that brain maturation is not only affected by significant interaction effects between sex and age but also by interactions between sex and measures of physical maturation (De Bellis et al. 2001) as assessed by Tanner stages (TS, Tanner and Davies 1985). It is believed that the development of GM volume in particular more or less coincides with the onset of puberty (De Bellis et al. 2001; Sowell et al. 2002; Gogtay et al. 2004). The increase of GM volume at the onset of puberty might reflect a coincident wave of synaptic proliferation and the gradual decrease in GM density that takes place after puberty might be attributed to postpubescent synaptic pruning (Bourgeois et al. 1994).

The remodeling of the adolescent brain is accomplished through a variety of mechanisms, including both progressive events such as increases in cell number, dendritic elaboration and axonal sprouting, and regressive events like apoptosis and synaptic pruning. These processes are known to be influenced by both androgens (MacLusky et al. 2006) and estrogens (EST) (Galea et al. 2006). The effects of sex steroids on brain morphology are classically described as operating through 2 distinct mechanisms (Cooke et al. 1998). One mechanism, called "organization," is defined as a developmental mechanism in which steroids act during critical periods to mediate permanent sexually dimorphic differentiation of brain morphology that gives rise to male and female sexual behavior and physiology in adulthood. The other mechanism, called "activation," is mediated through the acute effects of gonadal

hormones on the fully developed nervous system and is responsible for maintaining sex-specific behaviors in adulthood (Breedlove and Hampson 2002). Although the organization–activation framework for steroid control of reproductive behavior originally presumed a strictly activating role for gonadal steroids during adolescence, a recent modernization of this thinking incorporates dual roles for steroid hormones, proposing that they not only activate but also organize neural circuits during adolescence (Romeo et al. 2002). The sequence of events during steroid-dependent adolescent maturation of reproductive behavior may be an initial reorganization of circuits that further sensitizes them to hormone activation (Sisk and Foster 2004).

To date, however, there are no empirical studies in humans which directly link sex-specific changes in brain development to the general status of pubertal development and the effects of pubertal hormones in particular. Most of the available evidence has been derived either from animal studies (Nunez et al. 2001), studies of sex hormone changes during the menstrual cycle (Hausmann 2005), or studies on abnormal brain development in anomalous hormone or sex chromosome profiles (Giedd et al. 2006).

Thus, one challenge for developmental neuroscience is to identify which aspects of adolescent brain development are related to hormone levels and which are not (Giedd et al. 2006) and to understand the behavioral consequences of steroid-dependent organization and activation of the adolescent brain (Golubchik et al. 2007; Sato et al. 2008). Importantly, such knowledge of the mechanisms behind sexual differentiation of the brain might also contribute to a better understanding of the brain's susceptibility to neuropsychiatric disorders such as Attention Deficit Hyperactivity Disorder (ADHD), tics, eating disorders, and depression or schizophrenia, all of which show sex-specific prevalence rates, times of first manifestation, and courses. Data from animal studies suggest that derived sex differences in behavior are established during development by the actions of gonadal steroid hormones (Arnold and Gorski 1984). For example, odor preferences, behavioral responses to sensory stimuli, and social affiliations all change with adolescent development (Fleming and Corter 1995; Romeo et al. 1998). In addition, it has been shown that androgen deprivation causes a 40% decrease in synaptic density in the hippocampus of both rats and monkeys, and testosterone (TEST) replacement in male animals normalizes synaptic density (Leranth et al. 2003). Behaviorally, androgen deprivation by gonadectomy in male rodents impairs performance on tasks that depend on the hippocampus, such as maze learning and fear conditioning (Edinger and Frye 2004). In contrast, synapse number and dendritic spine density in the hippocampus of female rats seems to vary across the estrous cycle with low levels of estradiol being associated with lower synapse density and high estradiol levels being correlated with a higher density of synapses (Gould et al. 1990; McCarthy and Milner 2003).

However, some sexual dimorphisms are not completely explained by hormonal mechanisms (De Vries et al. 2002; Arnold 2004). For example, it has been demonstrated that the adolescent remodeling of cortical and subcortical regions also involves changes in synpatic organization at both pre- and postsynaptic levels which might contribute to regional differences in GM development. Andersen et al (2002) showed that prepuberty is characterized by a high level of expression of dopamine receptors in the striatum of the rat, in contrast to receptor pruning during the postpubertal stage. This pattern is more pronounced in males than in females, but in both sexes, it proceeds even when the gonads are removed before puberty suggesting that neither the overexpression nor the pruning of the dopaminergic receptors in the striatum are dependent on pubertal hormones.

Identifying the structural correlates of behavioral maturation and determining which structural features are related to steroid hormonal changes is an important area for further research. Accordingly, the aims of the present study were 1) to investigate sex differences in brain development during childhood and adolescence using fully automated voxel-based morphometry (VBM) and to discover which of these differences are influenced by sexual maturation and which are associated with the circulating level of steroid hormones and 2) to identify brain regions which are affected by circulating steroid levels using a whole-brain approach. Based on previous findings, we expected an enlargement of the amygdala in males and larger hippocampal and striatal GM volumes in females (Giedd et al. 1997). In addition, we hypothesized that amygdala and hippocampal volumes would be associated with sexual maturation as well as TEST and EST levels, whereas striatal volumes would be unaffected by circulating steroid hormones (Andersen et al. 2002). By combining direct (circulating hormonal levels) and indirect measures (TS) of puberty, we expect to get a better insight into sexual dimorphisms in brain development during childhood and adolescence.

Materials and Methods

Subjects

Forty-six normal subjects between 8 and 15 years of age (23 boys and 23 girls, for sample characteristics, see Table 1) were recruited as part of an ongoing neurodevelopmental study at the Department of Child and Adolescent Psychiatry. All subjects were carefully screened for childhood psychiatric disorders and neurodevelopmental diseases by a standardized semi-structured interview for the diagnosis of mental disorders in children (Unnewehr 1995). Intelligence quotient (IQ) was estimated on the basis of a short version of the Wechsler Intelligence Scale for Children III (Wechsler 1991; Tewes et al. 1999). Children with an IQ below 80 were excluded. The physical level of sexual maturation was assessed via TS, a 5-point scale describing the developmental state of primary and secondary sexual organs, in a medical examination conducted by an experienced physician. None of the subjects fulfilled the criteria for "pubertas praecox" or "pubertas tarda," and none of them were taking any medications at the time of the study, including oral contraceptives. Boys and girls did not differ significantly with respect to age, TS or IQ (see Table 1). All subjects were screened for laterality with the laterality score (LS) of the Edinburgh inventory (Oldfield 1971).

Except for 2 girls, all subjects were diagnosed as right handed (M_{LS} = 84, SD = 12). Within the sample of 46 subjects, TS correlated significantly with age (r = 0.87, P < 0.001). Children with TS = 1 or TS = 2 were classified as pre-early pubertal (n = 29, M_{age} = 10.3 years) and those with TS $\geq$ 3 (n = 17, M_{age} = 14.3 years) were classified as mid-late pubertal (Forbes et al. 2004).

Within this sample, 16 children and adolescents refused the collection of blood samples. Therefore, in a subsample of 30 subjects (15 girls and 15 boys, see Table 1), blood samples were obtained to determine levels of gonadotropins (FSH, follicle-stimulating hormone; LH, luteinizing hormone), gonadal steroid hormones (EST and TEST), and growth hormone (GH). Boys had significantly higher levels of TEST than girls, while sex differences for all other hormones were not significant (see also Table 1). Note, however, that a large number of subjects were classified as TS 1 or TS 2. After correcting the alpha level for multiple testing, TS correlated significantly with TEST

Table 1
Descriptive data of the whole sample (n = 46) and the subsample for the analysis of hormonal data (n =30)

	Mean	SD	Minimum	Maximum	Mean	SD	Min	Max	t	p_{corr}
Sample	23 boys				23 girls					
Age	11.70	2.28	8.00	15.09	10.92	2.06	8.01	15.03	1.23	0.14
TS	2.39	1.31	1.00	5.00	2.00	1.28	1.00	5.00	1.03	0.19
IQ	107.12	14.50	85.40	133.00	103.57	13.69	80.00	133.00	0.86	0.3
GBV/height	20.22	2.5	16.50	25.24	17.1	1.9	14.06	19.90	4.60	<0.01**
Subsample	15 boys				15 girls					
TS	2.60	1.30	1	5	2.07	1.48	1	5	1.05	0.41
EST	46.00	27.28	37.00	140.00	78.93	76.92	37	263.00	0.13	0.90
TEST	4.77	5.71	0.40	15.10	0.94	0.45	0.30	1.70	2.59	0.15
LH	2.09	2.93	0.30	11.70	1.45	2.50	0.30	9.80	0.65	0.52
FSH	1.91	1.25	0.40	5.30	2.69	1.76	0.40	6.00	1.41	0.17
GH	1.47	3.29	0.10	11.6	3.55	4.14	0.10	12.60	−1.52	0.14

Note: *$P < 0.05$; **$P < 0.01$. SD, standard deviation; EST, estradiol. Sensitivity and measurement ranges were EST (E2–6) 10–1000 pg/ml, TEST 10–1500 ng/dl, LH 0.07–200 mIU/ml, FSH 0.3–200 mIU/ml, GH 0.1–20 μg/l. Intra-assay variability ranges, depending on the mean VC, FSH = 2.0–2.9% VC, LH = 1.5–2.9% VC, EST = 4.0–12.1% VC, and TEST = 2.3–6.2% VC. Retest reliability scores were r_{EST} = 0.99, r_{TEST} = 1, r_{LH} = 0.99, r_{FSH} = 0.99, and r_{GH} = 0.99.

(r = 0.71, p_{corr} < 0.001), FSH (r = 0.57, p_{corr} = 0.001), and LH (r = 9.75, p_{corr} < 0.001) but not with EST (r = 0.46, p_{corr} = 0.15) or GH (r = –0.46, p_{corr} = 0.26) within the whole sample. The pituitary hormone LH correlated significantly with EST (r_{EST} = 0.75, p_{corr} < 0.001) and TEST (r_{TEST} = 0.603, p_{corr} < 0.001), whereas FSH did not (r_{EST} = 0.40, p_{corr} = 0.18; r_{TEST} = 0.40, p_{corr} = 0.16). All correlation coefficients followed the expected direction, and none of the hormone levels were significantly associated with global brain volume (GBV). In addition, significant associations were found between age and TEST (r = 0.71, p_{corr} < 0.001) and LH (r = 0.65, p_{corr} < 0.001) but not with EST (r = 0.27, P = 0.5) or FSH (r = 0.44, p_{corr} = 0.16).

This study was approved by the Medical Ethics Committee of the University Hospital of Aachen, and all volunteers and their parents gave informed consent.

Hormonal Assays

Blood samples were collected between 09.00 and 10.30 AM after the subjects had had a light breakfast in the morning. In postmenarchal girls (n = 5), blood samples were collected in the early follicular phase (days 2–7). All children were investigated between August and September to exclude seasonal effects on circulating level of hormones.

Serum was extracted from blood samples by centrifugation and stored immediately at –70 °C until analysis. Serum samples of all 30 subjects were analyzed using the ADVIA-Centaur System (1998, Bayer HealthCare, Leverkusen, Germany), a competitive immunoassay under the application of the direct chemiluminescent procedure. Sensitivity and measurement ranges were EST (E2–6) 37–1000 pg/ml, TEST 10–1500 ng/dl, LH 0.07–200 mIU/ml, FSH 0.3–200 mIU/ml, and GH 0.1–20 μg/l. The intra-assay variability, as measured by the range of the mean volume concentration (VC) was from 2.0% to 2.9% VC for FSH, from 1.5% to 2.9% VC for LH, from 4.0% to 12.1% VC for EST, from 2.3% to 6.2% VC for TEST, and from 2.01% to 4.12 VC% for GH; retest reliability scores were r_{EST} = 0.99, r_{TEST} = 1, r_{LH} = 0.99 r_{FSH} = 0.99, and r_{GH} = 0.99. Individual hormone levels of all subjects were normal according to the age-dependent standardized norms (for detail, see Table 1). Individual screening of gonadal hormones revealed that all cycling girls were in the targeted cycle phase. If a measured hormone concentration was below the sensitivity of the assay, it was expressed as the detection limit. Because some of the hormonal data were not normally distributed, nonlinear transformations (ln10) were applied prior to subsequent analyses.

MRI Data Acquisition

Magnetic resonance imaging was performed on a 1.5 T scanner SONATA MRI system (Siemens, Erlangen, Germany). Structural images were acquired for each subject using an isotropic T_1-weighted magnetization-prepared rapid acquisition gradient echo. Images yielded 160 contiguous isotropic 1-mm sagittal slices (image matrix = 180 × 256 mm^2, field of view = 256 mm, time repetition = 2200 ms, time echo = 3.93 ms, flip angle P = 15°, interslice gap = 0.5 mm).

Template Construction

In order to optimize the normalization procedure, a customized template was constructed from the data of the entire group of subjects (Wilke et al. 2003). Therefore, all structural images were segmented and further processed with linear and nonlinear spatial normalization steps. Mean images were calculated for all T_1 images as well as GM segments. Each resulting mean image was finally smoothed with an 8-mm full width half maximum (FWHM) Gaussian kernel, and these smoothed images then served as the final template for the subsequent preprocessing steps (for methods, see Good et al. 2001).

Data Preprocessing

All calculations and image manipulations were performed using MATLAB 6.5 (The Mathworks Inc., Natick, MA) and SPM2 (Statistical Parametric Mapping software, SPM; Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk). Structural data were analyzed via an optimized VBM procedure (Specht et al. 2005), which compares neuroanatomical differences on a voxel-by-voxel basis and which included segmentation, spatial normalization, and modulation processes. Spatial normalization inherently reduces size variations between different brains. To restore original volume information within each voxel, voxel values in the segmented images were modulated (multiplied) by the Jacobian determinants derived from the spatial normalization step. The analysis of modulated data, therefore, allows direct testing for regional differences in the absolute amount of each tissue type. Modulated GM segments were finally smoothed at 10-mm FWHM Gaussian kernel.

Statistical Analysis

Data were analyzed using a general linear model in SPM2. To identify sexually dimorphic brain areas, sex differences were calculated within an analysis of covariance (ANCOVA) model using GBV as a covariate of no interest. Individual GBV scores were determined for each subject as the sum of gray and WM volumes. For the identification of sex differences, region of interest (ROI) analyses were performed in regions reported to be sexually dimorphic in previous studies: the amygdala, the hippocampus, caudate nucleus, globus pallidus, and putamen (Caviness et al. 1996; Giedd et al. 1997; Lange et al. 1997).

In a second step, the effects of puberty (as assessed by TS and circulating hormonal levels) on sexual dimorphic brain regions were analyzed. Dependent variables were the intensity values of the local maxima extracted from modulated images and related to total brain volume to correct for GBV (% GBV). Within the sample of 46 subjects, univariate analysis of variance models were used to evaluate differences in the intensity values between TS and followed by Ryan–Einot–Gabriel–Welsch tests in case of significant F values. Within the subsample of 30 subjects with available hormonal data, the 5 TS were reduced to 2 conditions: pre-early (TS = 1–2) and mid-late puberty (TS ⩾ 3) according to Forbes et al. (2004). This procedure resulted in 2 groups of subjects ($n_{pre\text{-}early}$ = 16, aged from 8 to 12 years with mean TS = 1.2

and $n_{\text{mid-late}} = 14$, aged from 11 to 15 years with mean TS = 3.7). Main effects of sex, pubertal stage, as well as sex by TS interactions were calculated. Finally, linear regression analyses were conducted in order to determine which hormonal values best predicted the regional GM volume differences between the sexes. Bonferroni corrections for inflated Type I error were used in order to control for the number of analyses conducted. Effects were regarded as significant if they passed a corrected threshold of $P < 0.05$.

In order to analyze the effects of circulating TEST and EST levels across the whole brain, multiple regression analyses were conducted including hormonal levels as regressors of interest as well as GBV and age as regressors of no interest within the subsample of 30 subjects. Regressors were made orthogonal in order to satisfy statistical constraints (Büchel and Friston 1997). In case of significant interaction effects between sex and circulating steroid levels, further regression analyses were conducted for each sex group separately at that specific brain regions using a ROI approach (10-mm sphere centered at the local maxima derived from the whole-group analyses). For these more explorative analyses, whole-brain analyses were performed and results are reported as statistically significant at a level of $P < 0.05$, corrected for multiple comparisons on the cluster level with an underlying threshold on the voxel level of $P < .001$. Coordinates were reported in Montreal Neurological Institute (MNI) space.

Results

Sex Differences in GM Volumes and the Effects of Puberty and Circulating Steroid Hormones

Boys showed a significantly larger GBV of about 11% ($M_{23\text{boys}} = 1293 \pm 80.3\ \text{cm}^3$) than girls ($M_{23\text{girls}} = 1148.3 \pm 79.6\ \text{cm}^3$). The difference in GBV remained statistically significant after correction for body height differences (see Table 1). Morphometrical sex differences were therefore analyzed within ANCOVA models using GBV as a covariate of no interest. Boys exhibited larger GM volumes in the left amygdala. In contrast, girls showed higher GM volumes bilaterally in the tail of the hippocampus and the right striatum (caudate nucleus, globus pallidus, and putamen; see Table 2).

In order to disentangle the impact of pubertal stage and hormonal changes on regional GM volumes, intensity values of local maxima from sexually dimorphic areas were extracted and related to TS in the sample of 46 subjects. Within the sexually dimorphic area in the amygdala, regional GM volume varied by TS ($F = 3.5$, $P < 0.05$) and amygdala volume was significantly larger in TS 4 and 5 compared with TS 1–3 ($P < 0.05$) (see Fig. 1 and Table 2). This effect was also confirmed in the subsample of 30 subjects, indicating larger GM volume in the amygdala in mid-late pubertal subjects compared with pre-early pubertal children. No significant interaction between sex and TS emerged. In addition, linear regression analysis revealed that TEST (beta = 0.39, $P = 0.03$) but not EST levels ($P = 0.8$) predicted the intensity value within the amygdala ($R^2 = 0.15$; $P = 0.03$). Although the interaction effect between sex and circulating TEST level was found to be nonsignificant ($F = 2.2$), on a descriptive level, the scatter plot revealed that the higher TEST levels of older boys seem to be specifically associated with an increase in amygdala volume (see Fig. 1*C*).

In addition, the larger GM volumes of the posterior parts of the left hippocampus of girls varied as a function of TS ($F = 3.1$, $P < 0.05$): hippocampal volume was significantly larger in TS 1 than in TS 4 or 5 ($P < 0.05$) (see Fig. 2). Mid-late pubertal subjects showed significantly smaller GM volumes in the left hippocampus compared with pre-early pubertal boys and girls. Linear regression revealed a significant impact of circulating

Table 2
Sex differences as derived from ROI analyses

Brain region	Side	Coordinates				Main effect of Sex		Main effect of TS		Sex × TS Interaction		Main effect of Steroids		Sex × Steroid interaction	
		x	*y*	*z*		F/*P* value		F/*P* value		F/*P* value		F/*P* value		F/*P* value	
Amygdala	Left	−24	−9	−13	$N = 46$	$F_{(1,44)} = 6.8$, $P < 0.01$, boys > girls		$F_{(4,41)} = 3.5$, $P < 0.05$ TS 4–5> TS 1–3		$F_{(4,41)} = 1.6$, n.s.					
					$N = 30$	$F_{(1,\ 28)} = 4.9$, $P < 0.05$, boys > girls		$F_{(1,\ 28)} = 5.7$, $P < 0.05$ mid-late > pre-early		$F_{(1,28)} = 0.5$, n.s.		$F_{(1,\ 28)} = 4.3$, $P < 0.05$ TEST(beta = 0.39, $P < 0.05$)		$F_{(3,26)} = 2.2$, n.s.	
Hippocampus	Right	13	−33	9	$N = 46$	$F_{(1,44)} = 7.1$, $P < 0.01$, boys < girls		$F_{(4,41)} = 2.1$, $P < 0.1$		$F_{(4,41)} = 1.1$, n.s.					
					$N = 30$	$F_{(1,28)} = 5.7$, $P < 0.01$, boys < girls		$F_{(1,\ 28)} = 4.9$, $P < 0.05$, mid-late < pre-early		$F_{(1,28)} = 0.9$, n.s.		$F_{(1,28)} = 1.6$, n.s.		$F_{(3,26)} = 1.5$, n.s.	
Hippocampus	Left	−12	−34	8	$N = 46$	$F_{(1,44)} = 5.9$, $P < 0.05$, boys < girls		$F_{(4,41)} = 3.1$, $P < 0.05$, TS 1 > TS 4 and 5		$F_{(4,41)} = 0.93$, n.s.					
					$N = 30$	$F_{(1,28)} = 4.2$, $P < 0.05$, boys < girls		$F_{(1,28)} = 3.2$, $P < 0.05$, mid-late puberty < pre-early		$F_{(1,28)} = 0.3$, n.s.		$F_{(1,28)} = 4.2$, $P < 0.05$ TEST(beta = −0.36, $P < 0.05$)		$F_{(3,26)} = 1.8$, n.s.	
Pallidum	Right	28	−13	−1	$N = 46$	$F_{(1,44)} = 4.3$, $P < 0.05$, Boys < girls		$F_{(4,41)} = 1.5$, n.s.		$F_{(4,41)} = 0.4$, n.s.					
						$F_{(1,28)} = 4.0$, $P < 0.05$, boys < girls		$F_{(1,28)} = 0.8$, n.s.		$F_{(1,28)} = 0.3$, n.s.		$F_{(1,28)} = 0.3$, n.s.		$F_{(3,26)} = 1.7$, n.s.	
Caudate	Right	15	24	−9	$N = 46$	$F_{(1,44)} = 4.1$, $P < 0.05$, Boys < girls		$F_{(4,41)} = 1.5$, n.s.		$F_{(4,41)} = 0.6$, n.s.					
						$F_{(1,28)} = 4.06$, $P < 0.05$, boys < girls		$F_{(1,28)} = 0.6$, n.s.		$F_{(1,28)} = 1.2$, n.s.		$F_{(1,28)} = 0.3$, n.s.		$F_{(3,26)} = 1.7$, n.s.	
Putamen	Right	30	−16	−1	$N = 46$	$F_{(1,44)} = 4.1$, $P < 0.05$, Boys < girls		$F_{(4,41)} = 0.5$, n.s.		$F_{(4,41)} = 0.4$, n.s.		$F_{(1,28)} = 1.4$, n.s.		$F_{(3,26)} = 2.1$, n.s.	
						$F_{(1,28)} = 4.02$, $P < 0.05$, Boys < girls		$F_{(1,28)} = 0.8$, n.s.		$F_{(1,28)} = 0.3$, n.s.					

Note: n.s., not significant; EST, estradiol.

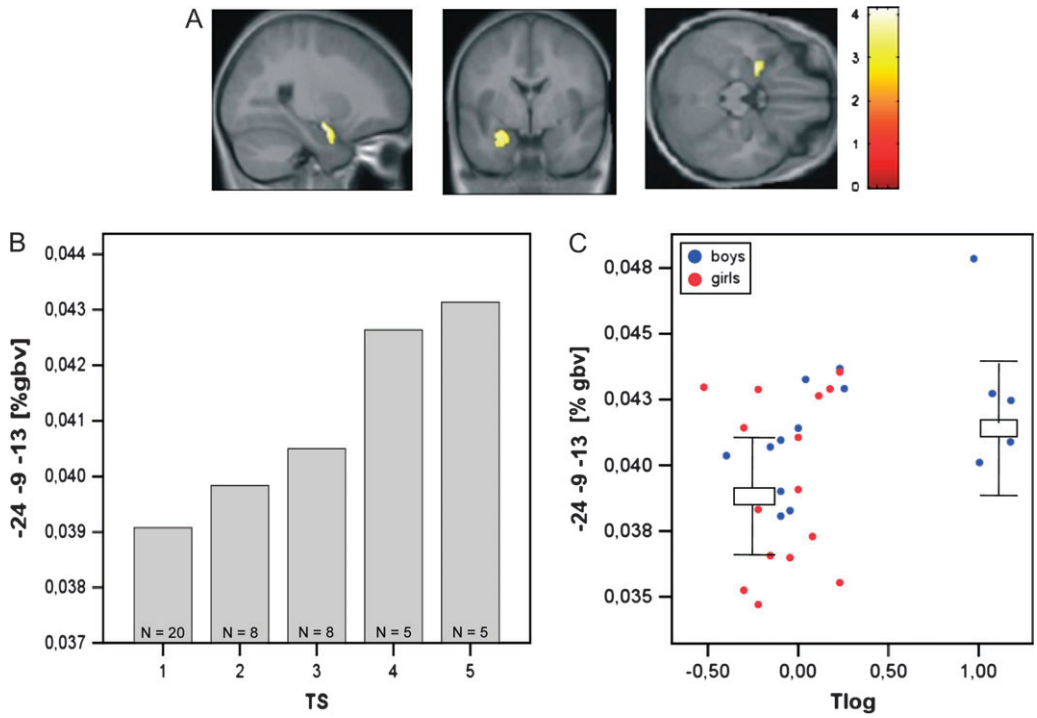

Figure 1. Regions of higher GM volumes in boys, resulting from MRI sex analysis. (*A*) Larger GM volume in the left amygdala in boys, thresholded at $P < 0.001$ on voxel level, corrected for multiple comparisons at $P < 0.05$ on cluster level, and overlaid on a mean structural image of the whole sample. Intensity values of local maxima (corrected for % GBV) as a function of TS (*B*) and ln TEST (*C*) for both sexes.

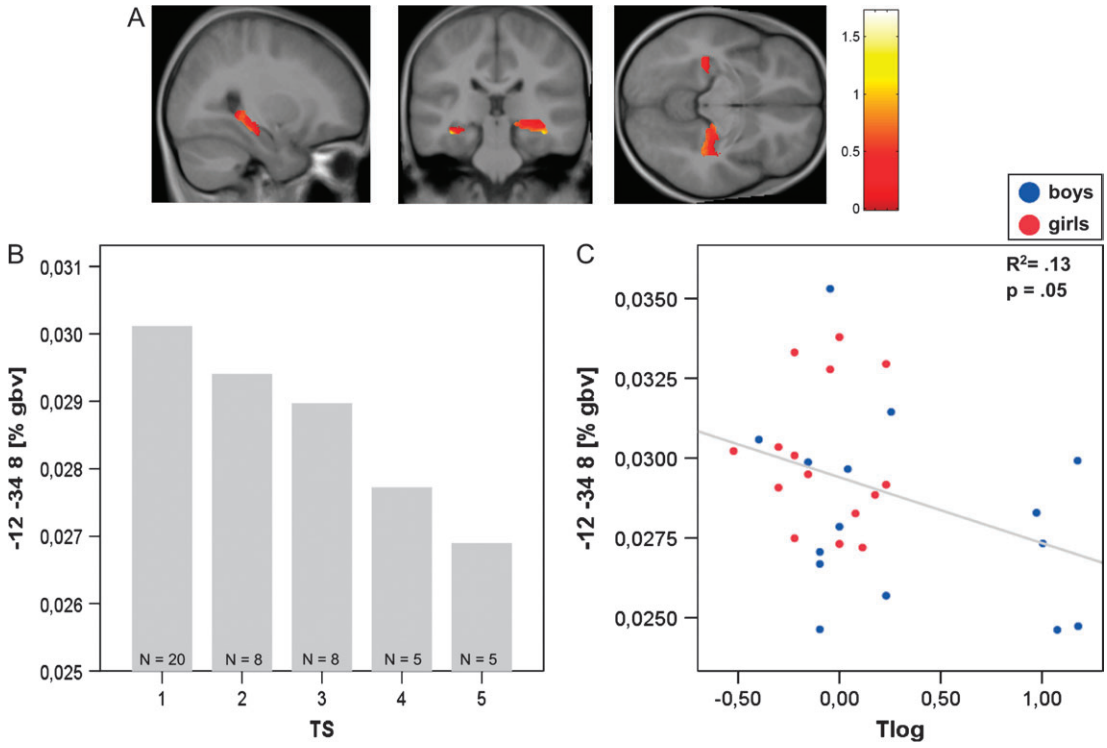

Figure 2. Regions of higher GM volumes in girls, resulting from MRI sex analysis. (*A*) Larger GM volume in the left hippocampal tail in girls, thresholded at $P < 0.001$ on voxel level, corrected for multiple comparisons at $P < 0.05$ on cluster level, and overlaid on a mean structural image of the whole sample. Intensity values of local maxima (corrected for % GBV) as a function of TS (*B*) and ln TEST (*C*) for the whole group.

TEST (beta = –0.36, $P = 0.05$) but not EST levels ($P = 0.8$) on hippocampal GM volume. Here, the intensity value in the left hippocampus significantly decreased when TEST increased (see Fig. 2*C*). Again, no significant interaction effects were found for sex by TS or sex by circulating hormonal levels in the hippocampus.

In contrast, striatal GM volumes did not vary as a function of TS (I > 0.9 for putamen, caudate, and pallidum) and neither TEST nor EST predicted the intensity values at the local maxima ($P > 0.8$).

Steroid Effects across the Whole Brain

In additional whole-brain regression analyses including circulating steroid levels as regressors and GM volume as dependent variable, EST levels were positively associated with GM volumes in the uncal cortex and parahippocampal gyrus bilaterally ($x_{right} = 12$, $y_{right} = 0$, $z_{right} = -30$, $Z_{right} = 4.2$, $x_{left} = -18$, $y_{left} = -6$, $z_{left} = -36$, $Z_{left} = 4.1$) across boys and girls. A significant sex × EST interaction effect was found for the intensity values at the local maxima bilaterally ($F_{right} = 4.9$, $P < 0.01$; $F_{left} = 4.7$, $P < 0.01$). The following sex-specific regression analyses revealed a particularly strong impact of circulating EST on right ($x = 13$, $y = 0$, $z = -29$, $Z = 3.9$) ($R^2_{\text{in girls}} = 0.187$; $R^2_{\text{in boys}} = 0.089$) and left ($x = -18$, $y = -7$, $z = -30$, $Z = 3.8$) ($R^2_{\text{in girls}} = 0.164$; $R^2_{\text{in boys}} = 0.061$) limbic brain structures in girls (see Table 3 and Fig. 3).

In addition, a positive association was found between circulating TEST levels and GM volumes in the right diencephalic structures, including the hypothalamus, mamillary bodies, and ventral thalamus ($x = 6$, $y = -11$, $z = -4$, $Z = 4.6$) extending to the left. GM volumes in the diencephalon increased with higher levels of circulating TEST, in particular in boys (sex × TEST interaction effect: $F = 5.2$, $P < 0.01$, $R^2_{\text{in boys}} = 0.65$, $R^2_{\text{in girls}} = 0.09$).

By contrast, negative associations were found for circulating TEST levels and GM volumes in the left parietal cortex including the precuneus and superior parietal gyrus ($x = -9$, $y = -53$, $z = 47$, $Z = 5.1$) (see Table 3 and Fig. 3). Here, GM volume decreased with increasing TEST levels across boys and girls. Again, the sex by TEST interaction effect was significant ($F = 4.1$, $P < 0.05$) with boys showing a significantly larger TEST effect in this particular brain region compared with girls ($x = -13$, $y = -50$, $z = 43$, $Z = 3.9$) ($R^2_{\text{in girls}} = 0.26$; $R^2_{\text{in boys}} = 0.60$).

Discussion

This is the first study which links sexual maturation directly to brain morphometry in normally developing children and adolescents. In line with previous studies (Giedd et al. 1997; Nunez et al. 2001) on structural brain development, typical sex differences were found, including larger GM volumes in the amygdala and smaller striatal and hippocampal GM volumes in boys compared with girls. These sex-specific developmental differences in brain morphometry have been previously described in normally developing children in both cross-sectional (Sowell et al. 2001; Gogtay et al. 2004; Wilke et al. 2007) and longitudinal studies (Giedd et al. 1999, 2006).

Table 3

Hormonal effects on brain morphometry as derived from whole-brain analyses (multiple regressions, $n = 30$, 15 boys 15 girls)

Contrast	Brain region	Side	*k*	MNI coordinates			*Z* score
				x	*y*	*z*	
EST (+)	Uncal cortex, parahippocampal gyrus	Bilateral	1114	12	0	−30	4.18
			653	−18	−6	−36	4.14
Sex by EST: significantly stronger positive association in girls			1917	13	0	−29	3.85
			1714	−18	−7	−30	3.78
TEST (+)	Diencephalon	Right	5980	6	−11	−4	4.57
Sex by TEST: significantly stronger positive association in boys			6508	−2	0	1	4.69
TEST(−)	Precuneus, superior parietal gyrus	Left	37727	−9	−53	47	5.05
Sex by TEST: significantly stronger negative association in boys			5119	−13	−50	43	3.93

Note: In case of significant sex by steroid interaction, sex-specific findings are included in the table. $P < 0.05$, corrected for multiple comparisons on cluster level with an underlying threshold of $P < 0.001$ uncorrected on voxel level. EST, estradiol; (+) positive correlation, (−) negative correlation.

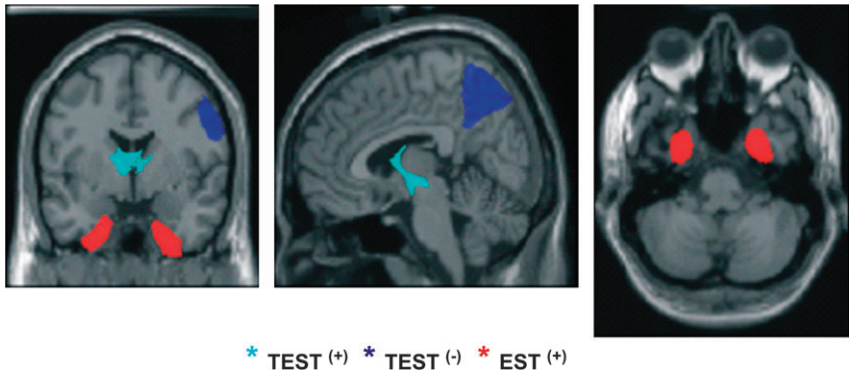

Figure 3. Impact of circulating steroid levels on GM volumes across boys and girls resulting from whole-brain regression analyses, thresholded at $P < 0.001$ on voxel level, corrected for multiple comparisons at $P < 0.05$ on cluster level, and overlaid on a mean structural image of the sex-specific group. Turquoise color represents positive TEST effects, blue color negative TEST effects, and red color positive EST effects on GM volumes.

Sexual dimorphism of brain structures may be related to sex chromosomes, hormonal effects, environmental effects, or a combination of these factors. Linear regression analyses revealed significant associations between pubertal development and sexually dimorphic brain areas in the amygdala and left hippocampus but not in the striatum. Gonadal steroid levels in children and adolescents explained 13–15% of regional GM volume variance in these specific brain regions. Thus, although there is clear evidence that sexual dimorphisms occur due to the organizing effects of sex hormones during prenatal development (for review, see Genazzani et al. 2007), our data suggest that gonadal steroid hormones also affect sexual dimorphisms later in life. Specifically, the present study showed a relationship between levels of gonadal steroid hormones and sex-dimorphic increases and decreases of regional GM volumes in the amygdala and hippocampus. Moreover, the combined analysis of TS and circulating hormonal levels might help to determine more precisely in which specific phase of pubertal development regional GM volume differences occur. It may also give first hints to whether small changes in hormonal secretion are related to brain development or only more dramatic hormonal changes as typically observed at the end of puberty impact have these effects. Thus, we were able to demonstrate that both amygdala and hippocampal volumes varied as a function of pubertal development in both sexes. The increase in amygdala volume did not occur before the end of puberty (TS 4 or TS 5) when adolescents showed increased circulating levels of TEST. In contrast, our data suggest that the larger hippocampal volume in girls might be associated with lower TEST in girls compared with boys during pubertal development. In addition, GM volumes in the medial temporal lobe/parahippocampal gyrus was positively associated with EST levels in particular in girls. This result is also interesting with regard to the neuroprotective properties of EST, as recently shown in a variety of in vitro and in vivo models of brain injury. Animal studies suggest beneficial effects of EST replacement therapy on cell death by suppressing apoptotic cell death pathways and enhancing the expression of genes that optimize cell survival (Wise 2006).

With regard to the organizational–activational framework (Sisk and Zehr 2005), our results suggest that changes in the level of circulating steroids during puberty mediate permanent sexual dimorphic differences in the amygdala–hippocampus complex in the human brain. These organizing effects become obvious in adolescents and are typically seen in the adult female and male brains (e.g., Filipek et al. 1994) but not in prepubertal children (Sowell et al. 1999). This brain maturation pattern might be further associated with a sensitization of the neural networks to hormonal activation such as an EST-dependent increase in brain activity in the amygdala–hippocampus complex during reward processing in adult women within the menstrual cycle (Dreher et al. 2007). However, longitudinal studies in humans are required to prove this hypothesis.

Our findings are in accordance with primate studies, which have shown that the amygdala predominantly contains androgen receptors, whereas other limbic brain structures such as hippocampal regions contain more EST receptors (Morse et al. 1986; Clark et al. 1988; Sholl and Kim 1989). However, we also found evidence for a relationship between TEST and a decrease in GM volume in the posterior hippocampus. Interestingly, Gogtay et al. (2006) recently demonstrated that the structural development of the hippocampus is indeed remarkably heterogenous. This is in line with our findings that both EST and TEST are associated with region-specific increases and decreases of GM volume within the neural circuitry of hippocampal and parahippocampal regions. Gogtay et al. (2006) described a greater loss of hippocampal volume at the posterior pole in females and at the head of the hippocampus in males. This finding is in contrast to our results of decreased GM volume in the posterior hippocampus in boys. However, note that sex differences were not tested directly in the study by Gogtay and colleagues. Interestingly, age-related changes in the functional organization of affective and memory circuits have also been observed in posterior hippocampal regions during adolescence (Nelson et al. 2003).

Our results are also in agreement with recent findings from studies on abnormal development. For example, clinical studies suggested that women with gonadal hypoplasia have decreased hippocampal volume (Murphy et al. 1993). In genetic syndromes, such as Klinefelter or Turner syndrome, androgens as well as ESTs seem to impact the volume of the superior and middle temporal gyrus (Nunez et al. 2001).

In addition, our results suggest that circulating TEST levels are also associated with GM volume in the parietal cortex in particular in boys. In a recent morphometric study, the most pronounced GM loss was found in the parietal lobes in normally developing children for both sexes (Wilke et al. 2007). Thus, the negative correlations between parietal structures and TEST in boys and girls might be considered within the context of the general decrease in parietal GM volumes during normal development. The development of GM in the parietal cortex might also be associated with its increasing specialization for visuospatial and attentional functions (Casey et al. 2005). This might indicate that neuronal cell death in these brain areas is directly associated with the circulating level of pubertal hormones. This is particularly interesting with regard to sex-specific differences in cognitive abilities, such as language or visuospatial skills. Behavioral studies have shown that performance in a mental rotation task improves significantly after a single injection of TEST in females, indicating a highly sensitive modulation of cognitive processes by circulating TEST in women (Hausmann et al. 2000; Aleman et al. 2004). Previous behavioral studies with healthy subjects also found associations between steroid levels and verbal skills (Gordon and Lee 1986). Our results did not reveal significant correlations between EST and GM volume in language-associated brain areas but supported an association between TEST and visuospatial skills mediated via parietal GM volume in children and adolescents in the age range of 8–15 years.

In addition, an association was observed between GM of diencephalic regions and TEST in boys. Diencephalic volumes have been reported in earlier developmental studies as regions which increase in size with age (Sowell and Jernigan 1998) in children and adolescents. In the early phase of puberty, relatively sudden increases in hormonal secretion of FSH and LH take place, which activate the gonadal production of steroid hormones. Thus, our data suggest that the increase in circulating levels of hormones might parallel a volume increase within the involved structures like the hypothalamus and the hypophysis gland and this might hint to a bidirectional relationship between circulating hormonal levels and brain structure/function in this particular brain region.

Boys were found to have smaller basal ganglia GM volume, which fits well with recent observations that, for example, caudate size peaks at age 7.5 in girls and at age 10.0 in boys. In line with our hypothesis, no significant association was found between pubertal development or circulating level of steroid and striatal GM volume. This is in accordance with the clinical observation that neuropsychiatric disorders are associated with striatal dysfunction. For example, ADHD and tic disorders are more frequently observed in boys than in girls. However, the typical age of onset of both neurodevelopmental disorders is clearly before puberty. This result is also in line with findings from rodent studies which have shown that the overexpression and subsequent pruning of striatal dopamine receptors is more pronounced in prepubertal males than females. However, neither process is dependent on pubertal gonadal hormones (Sisk and Zehr 2005).

In contrast, psychiatric diseases associated with primary dysfunction in limbic brain areas, such as mood and anxiety disorders, occur more often in females and typically occur during or after puberty. For example, it has recently been suggested that pubertal transition to TS 3 is associated with a sharp increase in depression rates in girls, with girls at TS 3 and higher being approximately 3 times more likely to be depressed than girls at TS 1 and 2 (Forbes et al. 2004; Patton et al. 2007). This fits well with the present findings of region-specific changes in limbic brain structures associated with EST and TEST during mid-late puberty. However, more recently it has been suggested that depression is also associated with functional abnormalities within the striatum (Epstein et al. 2006; Silverman et al. 2007). Interestingly, in contrast to findings of dysfunctional brain activity in a large limbic–cortical–striatal–thalamic network in early-onset major depression, the majority of anatomical studies did not find any persistent volume loss outside the hippocampus–amygdala complex (Hickie et al. 2005; MacMaster et al. 2008).

Thus, these data support the view that the timing of the interaction between structural brain development and the circulating level of pubertal hormones might affect the sex-specific risk for certain psychopathology during adolescence.

The major limitation of the present study is that it investigated a relatively small cohort, so the results must be interpreted with caution and should be replicated with larger samples. In addition, these data do not clarify whether hormonal changes alter neural circuits directly or whether puberty changes the social experience of adolescents, which may in turn influence brain development. In addition, non-hormonal genetic effects on brain development have to be taken into account. For example, Dewing et al. (2003) found sexually dimorphic patterns of gene expression in mice embryos and concluded that developmental differences between the brains of male and female mice were in part due to the differential expression of genes before gonadal secretion started. Other findings have suggested a genetic influence on the circulating level of GnRH, and hence a regulating effect of genes on puberty (Seminara et al. 2003; Navarro et al. 2004). Therefore, it would be interesting to combine genetic and hormonal analysis in future studies in order to disentangle more precisely hormonal and genetic effects on brain development during puberty. Despite these several limitations, the present study has directly linked pubertal stages and hormonal data to brain morphometry in normally developing children and adolescents. In agreement with previous studies in animals and humans, the results of the present study suggest that sexual maturation in general and circulating level of gonadal hormones in particular are specifically associated with regional GM differences in brain areas related to cognitive abilities and psychopathological vulnerabilities.

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Notes

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