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Sex steroids and brain structure in pubertal boys and girls

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Summary Sex steroids exert important organizational effects on brain structure. Early in life, they are involved in brain sexual differentiation. During puberty, sex steroid levels increase considerably. However, to which extent sex steroid production is involved in structural brain development during human puberty remains unknown. The relationship between pubertal rises in testosterone and estradiol levels and brain structure was assessed in 37 boys and 41 girls (10—15 years). Global brain volumes were measured using volumetric-MRI. Regional gray and white matter were quantified with voxel-based morphometry (VBM), a technique which measures relative concentrations ('density') of gray and white matter after individual global differences in size and shape of brains have been removed.

Results showed that, corrected for age, global gray matter volume was negatively associated with estradiol levels in girls, and positively with testosterone levels in boys. Regionally, a higher estradiol level in girls was associated with decreases within prefrontal, parietal and middle temporal areas (corrected for age), and with increases in middle frontal-, inferior temporal- and middle occipital gyri. In boys, estradiol and testosterone levels were not related to regional brain structures, nor were testosterone levels in girls. Pubertal sex steroid levels could not explain regional sex differences in regional gray matter density. Boys were significantly younger than girls, which may explain part of the results.

In conclusion, in girls, with the progression of puberty, gray matter development is at least in part directly associated with increased levels of estradiol, whereas in boys, who are in a less

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advanced pubertal stage, such steroid-related development could not (yet) be found. We suggest that in pubertal girls, estradiol may be implicated in neuronal changes in the cerebral cortex during this important period of brain development.

1. Introduction

Puberty is an episode in life which is characterized by hormonal fluctuations. During this period, there is a marked increase in sex steroids testosterone and estradiol, the end products of the maturing hypothalamus–pituitary–gonadal (HPG)-axis (Grumbach and Styne, 2003).

During puberty, sex steroids lead to the maturation of secondary sexual characteristics in a sex-specific manner (Grumbach and Styne, 2003). However, sex steroids also exert a wide range of effects on brain morphology, as the brain is an important target tissue for steroid hormone receptors (McEwen et al., 1982; McEwen, 1984). Among the important organizing effects of sex steroids is their influence on neurogenesis, receptor expression and neurite outgrowth (Romeo and McEwen, 2004). Most of these organizational effects take place during the prenatal period; however, these effects are subject to changing hormonal fluctuations throughout life and are not always irreversible (Pilgrim and Hutchison, 1994). Indeed, in adults, pharmacologically induced changes in levels of testosterone and estradiol have been shown to alter total brain and hypothalamus volumes (Hulshoff Pol et al., 2006).

During puberty, global gray (GM) matter volume within the cerebrum decreases (Giedd et al., 1999) and white matter (WM) volume increases (Giedd et al., 1999; Paus et al., 1999). Also, region-specific gray matter decreases have been reported in frontal, parietal and temporal areas (Jernigan et al., 1991; Giedd et al., 1999; Sowell et al., 2002, 2004; Gogtay et al., 2004; Wilke et al., 2007). It has been suggested that these neuro-anatomical changes during puberty and adolescence reflect the refinement of neuronal connections which could be related to cognitive and emotional development (Paus, 2005; Blakemore, 2008).

In keeping with the notion that puberty is a period of steroid-dependent brain organization (Romeo, 2003), the idea has been put forward that around the onset of puberty hormonal changes trigger selective neuro-anatomical alterations, as indirectly shown by gray matter decreases in frontal and parietal lobes (Giedd et al., 1999). Recently, in the early phase of puberty (at 9 years of age), elevated levels of the precursor of sex hormones, luteinizing hormone (LH) were found to be associated with cerebral white matter increases, whereas LH was not associated with gray matter (Peper et al., 2008). With the emergence of secondary characteristics of puberty (a result of sex steroid production), gray matter density decreases were found in frontal and parietal areas in 9-year-olds (Peper et al., in press). Thus, during early puberty, variation in pubertal mechanisms is accompanied by distinct structural brain changes. Interestingly, it was recently argued that the adolescent brain might respond differentially to changing steroid hormones levels over time (Sisk and Zehr, 2005) as brain development during puberty and adolescence is a dynamic and protracted process characterized by region-specific gray matter decreases and global white matter increases (Giedd et al., 2006). Consequently, it can be suggested that sex steroids testosterone and estradiol play a more prominent role in brain development in an advanced stage of puberty, compared to LH which is a marker of early puberty (Demir et al., 1996). In a first recent study on the interrelations between brain organization and sex steroid hormones in 30 children between 8 and 15 years of age, sex steroid levels were associated with sexual dimorphic gray matter areas (Neufang et al., in press).

The aim of the current study was to explore the interrelations between naturally occurring pubertal rises in testosterone and estradiol and brain structure in 10–15-year-old boys (37 subjects) and girls (41 subjects). As the progression of puberty is associated with global and regional gray matter decreases and white matter increases, it was expected that sex steroids are involved in these processes. The production of sex steroids occurs in a sex-specific manner. Also, the exposure to sex steroids has been implicated in the development and/or maintenance of sex differences in brain structure and these organizational effects of steroids are subject to changing hormonal fluctuations throughout life (Pilgrim and Hutchison, 1994). Therefore, we assessed the influence of sex steroids in boys and girls separately. Moreover, we explored to what extent pubertal rises of testosterone and estradiol levels are associated with sexually dimorphic brain areas.

2. Methods

2.1. Participants

The total sample consisted of 78 children between 10.0 and 14.9 years (Table 1), including 37 boys and 41 girls. These children are older siblings of twin-pairs which are described elsewhere (Peper et al., 2008, in press; van Leeuwen et al., 2008). Exclusion criteria consisted of any major medical or psychiatric illness and participation in special education. Parents and the participants themselves gave written informed consent to participate in the study. The study was approved by the Central Committee on Research involving Human Subjects (CCMO) of the Netherlands and was in agreement with the Declaration of Helsinki (Edinburgh amendments).

2.2. MRI acquisition

MRI scans were acquired from a 1.5 Tesla scanner (Philips, The Netherlands). A three-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head (256 × 256 matrix, TE = 4.6 ms, TR = 30 ms, flip angle = 30°, 160–180 contiguous slices; 1 mm × 1 mm × 1.2 mm voxels, field-of-view = 256 mm/70%) was acquired. Furthermore, a single-shot EPI (Echo Planar Imaging) scan was made as part of a diffusion tensor imaging (DTI)-series (SENSE factor 2.5; flip

Table 1 Demographics of the sample.

	Boys	Girls
N (individuals)	37	41
Age (mean, s.d.)*	11.6 (1.0)	12.2 (1.2)
Mean testosterone (pmol/l) (s.d.) (range)	70.8 (66.2) (15.4—285.6)	59.3 (35.7) (15.3—198.6)
Mean estradiol (pmol/l) (s.d.) (range)*	1027.0 (698.8) (249.4—3829.9)	2371.6 (1332.2) (719.5—6157.6)
Mean Tanner A (s.d.)*	1.6 (0.8)	2.9 (0.9)
Mean Tanner B (s.d.)*	1.5 (0.6)	2.8 (1.3)
Mean Tanner C (s.d.)	1.6 (0.7)	—
Mean height (s.d.)	152.7 (9.4)	156.0 (8.8)
Handedness (*R*/NR)	32/5	38/3

Age in years (s.d.); Tanner A = penis growth in boys (1—5); breast development in girls (1—5); Tanner B = pubic hair development (1—6); Tanner C = testis size (1—4); height in centimetres; handedness: *R* = number of right-handed; NR = number of non-right-handed. NB. Testosterone-levels were available in 29 boys and 38 girls; estradiol levels were available in 37 boys and 35 girls.

* Significant at $p < .05$ (corrected for age).

angle 90°; 60 transverse slices of 2.5 mm; no gap; 128 × 96 acquisition matrix; FOV 240 mm; TE = 78 ms) together with a magnetization transfer imaging (MTI) scan (60 transverse slices of 2.5 mm; no gap; 128 × 96 acquisition matrix; FOV 240 mm; flip angle 8°; TE = 4.5 ms; TR = 37.5 ms).

2.2.1. Volumetric processing

Our automatic image processing pipeline was used for segmentation of intracranial volume, cerebellum and global gray and white matter of the cerebrum. The T1-weighted images were automatically put into Talairach orientation (Talairach and Tournoux, 1988) without scaling, by registering them to a model brain in Talairach orientation. The two other scans were registered to the T1-weighted image by minimizing a mutual information joint entropy function (Maes et al., 1997). The co-registered scans were used for automatic segmentation of the intracranial volume, based on histogram analysis and morphology operations. The intracranial segment was subsequently checked visually and edited where necessary. The intracranial segment served as a mask for global gray and white matter segmentation. The software included histogram analysis, mathematical morphology operations, and anatomical knowledge-based rules to connect all voxels of interest, as was validated before (Schnack et al., 2001).

2.2.2. Voxel-based morphometry (VBM)

Regional measures of GM and WM concentration (''density'') were generated using voxel-based morphometry in a similar manner as was done previously (Peper et al., 2008, in press). VBM included the following steps. First, a model brain was created on a sample of 298 children aged 9—14 (including the 78 children discussed in this study), similar to the method used in Grabner et al. (2006). The use of a model brain specifically created from children's brains ensures an optimal warping from the individual brains to the model. Second, the binary GM and WM masks with voxels of 1 mm × 1 mm × 1.2 mm were blurred by a 3D Gaussian kernel (FWHM = 8 mm), in order to gain statistical power. The voxel values of these blurred GM and WM segments (between 0 and 1) reflect the local presence, or concentration, of GM or WM, respectively, and these images are referred to as ''density maps''. Third, in order to compare brain tissue at the same anatomical location in all subjects, the GM and WM segments were transformed into a standardized coordinate system (i.e. the model brain). These transformations were calculated in two steps. (A) The T1-weighted images were linearly transformed to the model brain. In this linear step a joint entropy mutual information metric was optimized (Maes et al., 1997). (B) Nonlinear (elastic) transformations were calculated to register the linearly transformed images to the model brain up to a scale of 4 mm (FWHM), thus removing global shape differences between the brains, but retaining local differences. For this step the program ANIMAL (Collins et al., 1995) was used. Fourth, the GM and WM density maps were transformed to the model space by applying the concatenated linear and nonlinear transformations. Finally, the maps were resampled to voxels of size 2 mm × 2 mm × 2.4 mm. Voxels with an average GM density below 0.1 were excluded from the GM density voxel-based analysis. Similarly, voxels with an average WM density below 0.1 were excluded from the WM density voxel-based analysis. Using 'non-modulated' VBM analyses allow for direct investigation of regional differences in brain areas without being confounded by overall brain size, i.e. these individual differences in brain size and shape have been removed by linear and nonlinear transformations.

2.3. Hormonal measurements

Free (bioavailable) testosterone levels were determined in first saliva (Competitive immunoassay (luminiscention), IBL Hamburg). The intra-assay and inter-assay coefficients of variation (CVs) were below 12% at the lower limit of detection of 11 pmol/l. Total estradiol levels were determined in first morning urine (Competitive immunoassay (luminiscention), Architect, Abbott Laboratories, Abbott Park, IL, USA). The intra-assay and inter-assay CVs were 5% and 10%, respectively, at levels > 150 pmol/l (lower limit of detection) and <9000 pmol/l (upper limit of detection). Urinary estradiol levels were divided by creatinine level to correct for variations in urine excretion rate.

Both testosterone and estradiol data were collected on two consecutive days at consistent times directly after waking up. Analyses were carried out by the endocrinological laboratory of clinical chemistry of the VU Medical Center in Amsterdam, the Netherlands. Via the enzyme aromatase, testosterone is (partly) converted into estradiol (see Collaer and Hines, 1995); therefore testosterone and estradiol are

analyzed in both boys and girls. The two consecutive testosterone and estradiol measurements were significantly correlated in both boys ($r = .70$, $p < .00001$ and $r = .47$, $p < .003$) and girls ($r = .69$, $p < .00001$ and $r = .60$, $p < .00001$). In the analyses, averages of the two measurements were used.

Secondary sexual characteristics of puberty were measured by a trained researcher using a Tanner questionnaire (no self-report) (Marshall and Tanner, 1969, 1970). The Tanner questionnaire consists of a six-category measurement of pubic hair in both boys (induced by androgens mainly from the testes) and girls (induced by androgens from both the adrenal gland and the ovaries). In girls, breast development, induced by estrogen secretion from the ovaries, is measured using five different stages ranging from 1 to 5. In boys, penis and testicle size (under androgenic control) are assessed based on scales divided in 6 (ranging from 1 to 6) and 4 (ranging from 1 to 4) stages, respectively. On all these scales, the lowest stage (1) represents pre-puberty and the highest stage corresponds to fully mature. A regular menstrual cycle was present in five girls (12%) and three more girls (7%) had had a menstruation only once at the moment they participated in our study (thus having an irregular cycle). None of the female participants used oral contraceptives. Testosterone levels were available of 29 boys and 38 girls, and estradiol levels in 37 boys and 35 girls: estradiol and testosterone samples were not collected from six children, whereas in five other children testosterone levels were below the detection limit of 11 pmol/l.

2.4. Statistical analysis

Data were examined for normality in girls and boys separately. In boys, the averages of the two measurements of testosterone and estradiol were not normally distributed (Kolmogorov Smirnov (KS) test: $p < .006$ and $p < .002$, respectively); therefore a log-transformation was applied leading to a normal distribution of testosterone and estradiol levels (KS-test: $p = .36$ and $p = .10$). In girls, mean testosterone and estradiol levels were normally distributed (KS-test: $p = .34$ and $p = .43$). However, to consistently analyse the data within boys and girls, testosterone and estradiol data in girls were also log-transformed (leading to the following KS-test results: $p = .64$ and $p = .91$, respectively). A possible explanation for the difference in distributions of hormonal data in boys and girls could be due to the presence of a larger number of boys with relatively low hormonal levels as compared to boys with relatively high levels. Within the group of girls, the number of high and low hormonal levels was more equally distributed.

The association between testosterone or estradiol and absolute global brain volumes (total brain, cerebellum and gray and white matter) was investigated with a (linear) regression analysis within boys and girls separately, corrected for age. Furthermore, to investigate whether the possible relation between sex steroids and global brain volumes could be attributed to total brain volume, the associations between sex steroids and cerebellum, gray and white matter volumes were subsequently corrected for total brain volume. To that end, gray and white matter and cerebellum volumes were calculated as proportions of total brain volume. It should be noted that in the current sample of children, total brain volume and intracranial volume were highly correlated ($r = .98$), therefore we chose to calculate relative global brain volumes as a proportion of total brain volume.

In the voxel-based analysis, a (linear) regression was carried out to estimate the effect of hormone level on regional gray and white matter density within boys and girls separately, corrected for age and handedness. *t*-Tests were performed to establish significance. A correction for multiple comparisons was carried out according to the false discovery rate (FDR; $\alpha = 0.05$, two-tailed) allowing for an average of false positives of 5% (Genovese et al., 2002). Sex differences in global brain volume and regional gray and white matter density were analyzed in boys and girls together. The effect of sex was estimated with a (linear) regression analysis, correcting for age. Also, the interaction between sex and age was investigated. In the voxel-based analysis, *t*-tests were performed to establish significance (FDR; $\alpha = 0.05$, two-tailed).

3. Results

3.1. Pubertal hormones and secondary sexual characteristics

On average, girls were older than boys ($F_{(1,77)} = 5.45$, $p < .02$) (Table 1). Moreover, girls were in more advanced stages of puberty (mean Tanner stage) ($F_{(1,77)} = 33.83$, $p < .0001$). Indeed, all girls showed development of secondary sexual characteristics, compared to 62% of the boys ($N = 23$). After correction for age on the total sample of children, testosterone levels were equal in boys and girls ($F_{(1,66)} = 1.06$, $p = .31$). Estradiol levels were higher in girls as compared to boys ($F_{(1,71)} = 32.20$, $p < .0001$). The difference in estradiol level between boys and girls and the equal level of testosterone in boys and girls remained unchanged without an age-correction (($F_{(1,71)} = 38.06$, $p < .0001$) and ($F_{(1,66)} = 0.11$, $p = .74$), respectively). Corrected for age, testosterone and estradiol levels were significantly correlated in girls ($r = .49$, $p < .005$), but not in boys ($r = .28$, $p < .15$). Without correcting for age, these correlations were 0.60 ($p < .001$) and 0.27 ($p < .15$), respectively.

Except for estradiol in boys, age was highly correlated with steroid levels in both sexes (r's $> .41$) (Table 2).

3.2. Associations between sex steroids and brain structures

3.2.1. Global brain volumes

Age was not significantly correlated with absolute brain volumes within both sexes. However, in girls, gray matter proportion (relative to total brain volume) decreased with age ($r = -.36$, $p < .02$) whereas white matter proportion increased ($r = .37$, $p < .02$). In boys, age and gray or white matter proportion were not significantly associated. In girls, after correcting for age, a higher estradiol level was correlated with a smaller absolute gray matter volume ($r = (.39$, $p < .03$) (Table 3 and Fig. 1), but not with gray matter proportion. Estradiol level did not correlate with global brain volumes in boys. After correcting for age, a higher level of testosterone in boys was correlated with a larger absolute gray matter volume ($r = .41$, $p < .03$) (Table 3) but not with

Table 2 Correlations between pubertal hormones, Tanner stages (Spearman's Rho) and age (Pearson's *r*).

	Estradiol		Testosterone	
	M	F	M	F
Tanner A	0.13	0.69*	0.48*	0.60*
Tanner B	−0.07	0.67*	0.26	0.63*
Tanner C	−0.06	–	0.32	–
Age	0.18	0.59*	0.59*	0.41*

M: male; F: female; Tanner A: penis growth in boys and breast development in girls; Tanner B: pubic hair development; Tanner C: testis size.
* Significant at $p < .01$.

Table 3 Associations between global brain volumes, age and sex steroids.

Volume	Age		Testosterone[a]		Estradiol[a]	
	M	F	M	F	M	F
Total brain	0.22	−0.12	0.32§	−0.06	0.13	−0.32§
Gray matter	0.10	−0.27§	0.41*	−0.11	0.20	−0.39*
White matter	0.27	0.11	0.17	0.04	−0.04	−0.17
Cerebellum	0.22	−0.09	−0.10	0.06	0.12	−0.21

M = male; F = female. Depicted are (partial) correlations (Pearson's *r*).
[a] Corrected for age.
* Significant at $p < .05$.
§ Significant at $p = .07$.

gray matter proportion. Testosterone was not associated with brain volumes in girls.

3.2.2. Regional gray and white matter density

Significant main effects of age were found in girls only, comprising gray matter decreases within the precentral gyrus bilaterally, superior temporal gyrus bilaterally, left angular gyrus, left middle frontal and right inferior temporal gyri (with *t*-values up to 7.24 (critical *t*-value of significance was 3.35)). After controlling for age and handedness, in girls a higher estradiol level was associated with a lower gray matter density in prefrontal (superior-, inferior- and orbitofrontal gyri), parietal (supramarginal and angular gyri) and middle temporal areas with *t*-values up to 5.77 (critical *t*-level of significance was 4.60) (Table 4 and Fig. 2a). For an illustration of the negative (partial) correlation between estradiol and gray matter density within the inferior frontal gyrus, see Fig. 3.

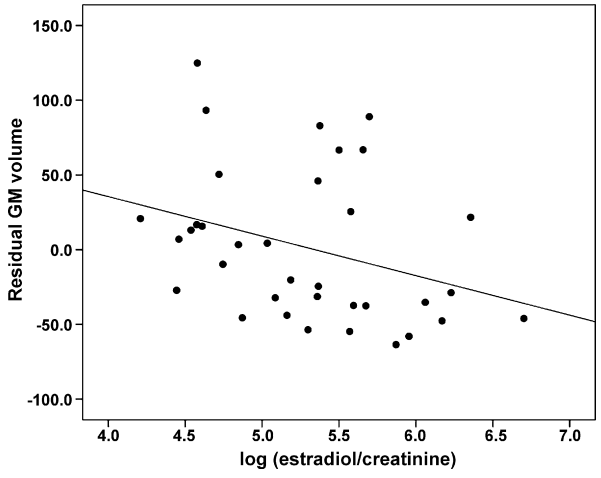

Figure 1 Estradiol associated with gray matter volume decrease ($N = 35$ girls), corrected for age. Values on the *y*-axis represent unstandardized residuals of gray matter volume, corrected for age. The corresponding correlation coefficient is − 0.39.

The reversed pattern (higher gray matter density with a higher estradiol level) could be observed in (parts of) the middle frontal-, inferior temporal- and middle occipital gyri with *t*-values up to 5.35 (critical *t*-level of significance was 4.60) (Table 4 and Fig. 2b). Estradiol levels were not associated with white matter in girls. In boys, estradiol level was not associated with regional gray or white matter density. Testosterone levels were not significantly associated with gray or white matter density, in boys or in girls, although in girls a trend was found for gray matter decreases in the right fusiform gyrus, left inferior frontal gyrus and left middle temporal gyrus with increased levels of testosterone ($t = 4.90$; at an FDR-value of $\alpha = 0.07$).

3.3. Sex differences in brain structures

3.3.1. Global brain volumes

After correcting for age, boys showed a larger (absolute) total brain volume ($F_{(1,77)} = 26.38$, $p < .000002$), cerebellum ($F_{(1,77)} = 4.99$, $p < .03$), white matter ($F_{(1,77)} = 22.39$, $p < .0001$) and gray matter ($F_{(1,77)} = 21.92$, $p < .00001$). There were no sex differences in relative cerebellum, global gray or white matter proportions.

3.3.2. Regional gray matter density

The critical *t*-value of significance, corrected for multiple comparisons (FDR, $\alpha = 0.05$) was 2.88. After correcting for age and handedness, boys showed increased gray matter density mainly in the right middle temporal gyrus, right inferior frontal gyrus, insular gyrus bilaterally, putamen bilaterally, left rostral anterior cingulate gyrus, hypothalamus, thalamus, globus pallidus, amygdala and left middle occipital gyrus with *t*-values ranging from 3.85 to 7.27 (Table 5 and Fig. 4a). Girls had a higher gray matter density in small parts of the right posterior hippocampus, right insula, right anterior caudate nucleus, left inferior frontal gyrus and left caudal anterior cingulate cortex with *t*-values ranging from 2.94 to 5.38 (Table 5 and Fig. 4b). The most pronounced sexual dimorphic areas (M > F) included the putamen, insula and amygdala with corresponding *t*-values of 6.85, 7.28 and 7.53, respectively.

There were no significant interactions between age and sex for gray and white matter densities.

4. Discussion

To our knowledge, this is the first study investigating interrelations between naturally occurring pubertal rises in testosterone and estradiol and brain structure in 10–15-year-old boys (37 subjects) and girls (41 subjects) separately. Gray

Table 4 Significant associations between estradiol level and gray matter density in girls ($N = 35$).

Area	t	r	Talairach coordinates		
			X	Y	Z
Increases					
Mid. Front. gyrus R	5.35	.69	35	19	47
Inf. Temp. gyrus R	4.65	.64	45	−7	−35
Mid. Occ. gyrus R	4.95	.66	39	−77	3
Decreases					
Inf. Front. gyrus L-Ant.	5.77	−.72	−40	52	3
Inf. Front. gyrus L-Post	5.25	−.65	−57	14	3
Sup. Front. gyrus L (R)	5.77 (4.90)	−.72 (−.66)	−11 (15)	42 (46)	50 (47)
Orbitofrontal gyrus-Post (L)	4.90	−.66	−26	42	−10
Mid. Front. gyrus L	4.81	−.66	−43	34	30
Supramarginal gyrus R (L)	5.33 (4.70)	−.69 (−.64)	65 (−60)	−31 (−35)	36 (37)
Mid. Temp. gyrus R	5.59	−.71	65	−29	−1
Angular gyrus L	5.59	−.71	−48	−56	47

Ant. = anterior; Front. = frontal; Inf. = inferior; L = left; Mid. = middle; Occ. = occipital; R = right; Sup. = superior; Temp. = temporal. Critical t-value according to FDR ($\alpha = 0.05$) was 4.60. r = partial correlation (corrected for age/handedness). Voxels with the highest t-values within significant brain areas are depicted.

matter volume decreases with age was observed in girls only. After correcting for age, in girls, a higher level of estradiol was associated with a smaller global gray matter volume, whereas in boys, a higher level of testosterone was associated with larger gray matter volumes. These effects on global gray matter were also in part driven by total brain volume. Regional age-related changes were observed in girls only, including gray matter decreases within the outside borders of middle frontal, angular gyrus of the parietal lobe, superior and inferior temporal gyri. Corrected for age, an increased level of estradiol in girls was associated with decreased gray matter densities in the superior-, inferior-, middle- (left) and orbitofrontal gyri, supramarginal and angular gyri of the parietal lobe and middle temporal gyrus. In girls, estradiol-related increases were associated with gray matter densities of the middle frontal (right), inferior temporal and middle occipital gyri. These estradiol-related brain areas did not show sex differences in density. Prominent regional sex differences in several brain structures were found in the amygdala, putamen, thalamus, insula, rostral

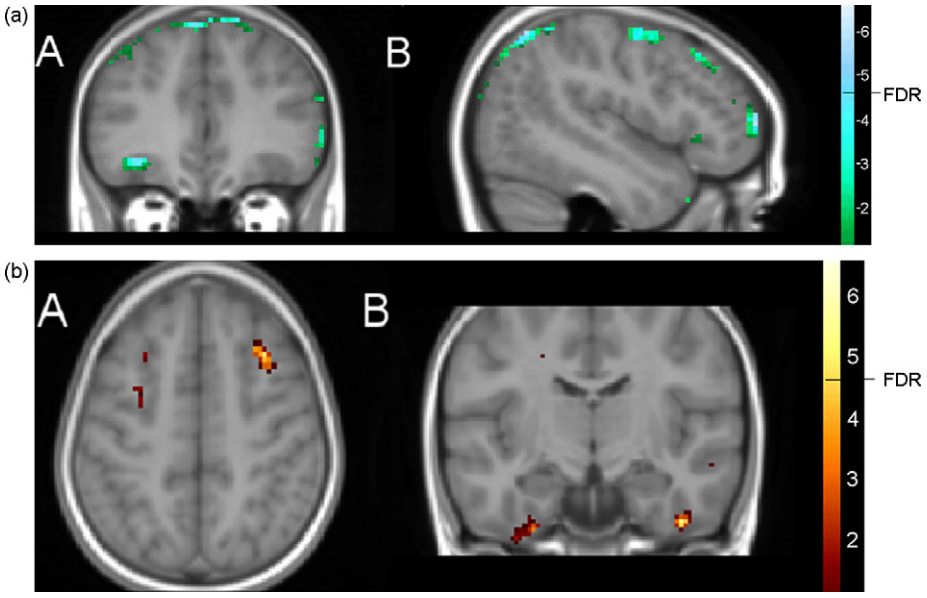

Figure 2 (a) Estradiol associated with gray matter decreases ($N = 35$ girls (10—15 years old)), corrected for age. Bilateral superior- and left orbitofrontal gyri (A), $Y = 42$, and right inferior frontal and angular gyri (B), $X = (48$. Images are according to neurological convention (left = left). Critical level of significance is −4.60, corrected for multiple comparisons according to the false discovery rate, $\alpha = 0.05$, two-tailed). Significant voxels are overlaid on our created model brain. (b) Estradiol associated with gray matter increases ($N = 35$ girls (10—15 years old)), corrected for age. Right middle frontal gyrus (A), $Z = 47$ and right inferior temporal gyrus (B), $Y = (7$. Images are according to neurological convention (left = left). Critical level of significance is 4.60, corrected for multiple comparisons according to the false discovery rate, $\alpha = 0.05$, two-tailed. Significant voxels are overlaid on our created model brain.

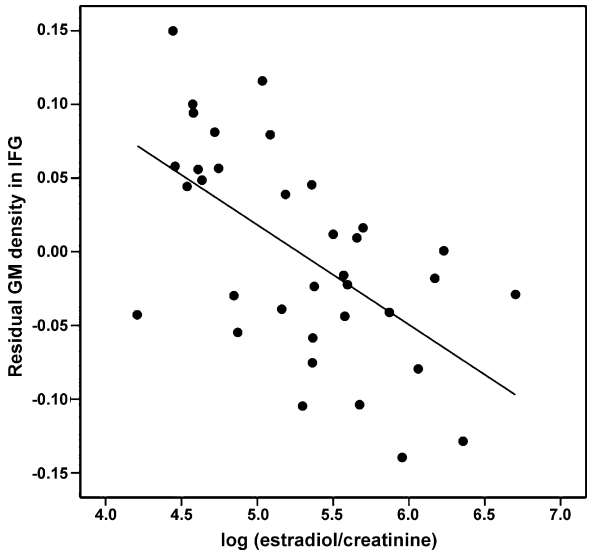

Figure 3 Estradiol associated with gray matter decrease within the inferior frontal gyrus ($N = 35$ girls). The graph represents the residual of gray matter density (corrected for age effect) within the left inferior frontal gyrus (IFG) (Talairach coordinates: −40, 52 and 3). The corresponding correlation is −0.72.

anterior cingulate, and superior temporal gyrus (larger in boys) and in the hippocampus, caudate nucleus, caudal anterior cingulate, middle temporal gyrus and inferior occipital gyrus (larger in girls). These areas did not show associations with pubertal sex steroid levels.

In earlier MRI studies it was reported that frontal and parietal gray matter decreases fastest over the course of adolescence contrary to other cortical brain areas (Jernigan et al., 1991; Giedd et al., 1999). Moreover, in the early pubertal stage, these areas were found to decrease with the emergence of secondary sexual characteristics (Peper et al., in press). In this study, in girls, we could indeed replicate these age-related gray matter decreases reported earlier (Jernigan et al., 1991; Giedd et al., 1999), whereas in boys we could not demonstrate significant age-related brain changes. Thus, endocrinologically, girls advance into puberty 1−2 years before boys, a phenomenon which has also been demonstrated with respect to peak gray matter volume in frontal and parietal areas (Giedd et al., 1999; Lenroot et al., 2007). Our data also indicate that at this age the process of gray matter decrease is already ongoing in girls as opposed to boys. Importantly, increased production of estradiol in girls seems to be directly related to the negative association with frontal and parietal gray matter. We also found a positive association with estradiol and gray matter density in temporal and occipital areas in girls. This is in agreement with findings that gray matter in these areas increases well into adolescence (Giedd et al., 1999; Lenroot et al., 2007).

In the present study, we used voxel-based morphometry to show gray matter density decreases that might indicate cortical thinning (Sowell et al., 2004). Decreases in gray matter during puberty have been suggested to reflect decreases in for example dendritic branching or number of synapses, however evidence from humans is currently lacking. In rats it was found that estrogen is involved in apoptotic processes, at least in the hippocampus (Barker and Galea, 2008). The first human neuroimaging study that has investigated the association between the precursor of sex hormones, luteinizing hormone and brain morphology in children at the onset of puberty (9 years of age), reported an association between increased LH-levels and cerebral

Table 5 Sexually dimorphic brain areas (gray matter density), measured in 78 children.

Area	*t*	Talairach coordinates		
		X	*Y*	*Z*
Boys > girls				
Inf. Front. gyrus R	6.27	56	−9	12
Insula L (R)	7.27 (5.90)	−38 (39)	−3 (−5)	13 (11)
Ant. cingulate − rostral L	6.53	−1	35	−6
Putamen L (R)	6.21 (4.58)	−28 (26)	−8 (7)	4 (3)
Amygdala L	7.58	−21	−10	−16
Thalamus L	5.21	−8	−27	5
Sup. Temp. gyrus L	3.85	−60	−9	3
Post. cingulate gyrus L	4.52	−2	−38	26
Girls > boys				
Inf. Front. gyrus R	3.41	41	37	10
Ant. cingulate −caudal L	4.48	−8	46	13
Hippocampus R	2.94	32	−29	−8
Caudate nucleus R	3.79	10	7	13
Mid. Temp. gyrus R	4.63	54	−2	−23
Sup. Temp. gyrus R	4.59	45	−28	15
Inf. Occ. gyrus L	5.38	−47	28	−14

Ant. = anterior; Front. = frontal; Inf. = inferior; L = left; Mid. = middle; Occ. = occipital; R = right; Sup. = superior; Temp. = temporal. Critical *t*-value according to FDR ($\alpha = 0.05$) was 2.88. Voxels with the highest *t*-values within significant brain areas are depicted. Analyses are corrected for age and handedness.

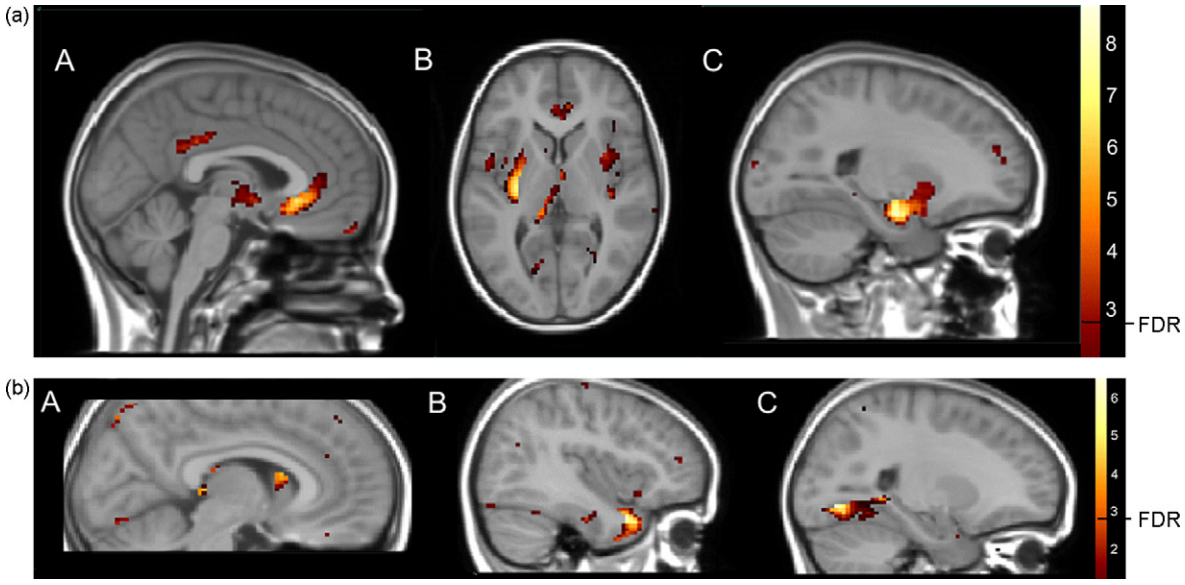

Figure 4 (a) Larger gray matter density in boys compared to girls between 10 and 15 years (corrected for age). (A) Left rostral anterior cingulate gyrus (X = (1), (B) left putamen (Z = 4), and (C) left amygdala (X = (21). Critical level of significance is 2.88, corrected for multiple comparisons according to the false discovery rate, α = 0.05, two-tailed. Significant voxels are overlaid on our created model brain. (b) Larger gray matter density in girls compared to boys between 10 and 15 years (corrected for age). (A) Right caudate nucleus (X = 10), (B) right superior and middle temporal gyri (X = 45), and (C) left inferior occipital gyrus (X = (47). Critical level of significance is 2.88, corrected for multiple comparisons according to the false discovery rate, α = 0.05 (two-tailed). Significant voxels are overlaid on our created model brain.

white matter increases, whereas LH was not associated with gray matter (Peper et al., 2008). In the current study we found that in more advanced pubertal girls, estradiol is related to gray matter and not to white matter. Thus, it might be speculated that selective neuro-anatomical properties mature in conjunction with the secretion of distinct HPG-axis hormones.

Recently, in the first study on sex steroid levels and brain morphology in 8—15-year-old children, an estrogen-related increase in the parahippocampal gyrus was found as well as a testosterone-related increase in diencephalic brain areas and a decrease in parietal gray matter, when males and females were analyzed together (Neufang et al., in press). We could not demonstrate testosterone-related associations with regional gray (or white) matter density in boys or girls separately. The discrepancies between our study and Neufang et al. (in press) could be due to a number of factors. Age might contribute to differences between the two studies since we included a sample of 10—15 years and Neufang et al. (in press) included also children between 8 and 9 years of age. Within the hormonal analyses, males and females were analyzed together, whereas we chose to analyse the sexes separately. Furthermore, we measured relative gray and white matter density, correcting for global volume and shape differences, whereas Neufang et al. (in press) report on modulated VBM measurements. Finally, our sample size allowed for a correction for multiple comparisons on voxel-level, whereas Neufang et al. (in press) applied a correction on cluster-level, which is less conservative.

There are studies which indicate that throughout life, neural circuits remain responsive to changes in circulating sex steroids: for example, in adults, pharmacologically induced changes in levels of testosterone and estrogen have been shown to alter total brain and hypothalamus volumes (Hulshoff Pol et al., 2006). In addition, estrogen replacement therapy in postmenopausal women causes enlargement of neocortical areas (Boccardi et al., 2006) and hippocampal volumes (Eberling et al., 2003; Boccardi et al., 2006; Lord et al., 2008) compared to postmenopausal women not using estrogen therapy. Furthermore, animal studies point to a direct role for estrogens in positively affecting neuronal properties, such as synaptic spine density (reviewed by Woolley, 1999). Finally, it was reported that estrogen could change neuronal gene expression in the primate prefrontal cortex, causing increases or decreases depending on type of gene transcription factor (Wang et al., 2004).

We were not able to show associations between sex steroid levels and regional brain structures in boys. An explanation might be that males' and females' brains respond differentially to effects of sex steroids: it has been reported that female rats were more responsive to the effects of estradiol on neurogenesis and apoptosis than male rats (Barker and Galea, 2008). Moreover, it can be argued that at this age, circulating sex steroid levels might not be sufficient to induce an effect on regional brain structures. Indeed, peak levels of testosterone production in boys are found at (genital) Tanner-stage 4 (Butler et al., 1989), which is, on average, reached around 13 years (Marshall and Tanner, 1970). Future research in more advanced pubertal boys, rising testosterone levels may indeed be found to be related to development of specific brain areas. For example, in structures with a high density of androgen receptors such as the amygdala, hypothalamus and several cortical areas

(Finley and Kritzer, 1999; Simerly et al., 1990) an association with testosterone can be expected.

The increased production of sex steroids at puberty was not related to any of the focal sexually dimorphic brain structures found at this age. However, the reported sexually dimorphic brain areas did overlap with areas found in adults (Chen et al., 2007; Goldstein et al., 2001, Good et al., 2001) and in children or adolescents (Durston et al., 2001; Giedd et al., 1997; Lenroot et al., 2007). Also, in various brain areas showing sexual dimorphisms at this age, androgen and estrogen receptors are present, including the hypothalamus, thalamus, amygdala, hippocampus (Simerly et al., 1990) and throughout the cortex (Finley and Kritzer, 1999; Simerly et al., 1990). The fact that we could not demonstrate a significant relation between sex steroids and sex differences in brain structure could be due to a number of factors. The influence of sex hormone exposure leading to sex differences in brain structure is possibly most pronounced during the pre/perinatal period than during puberty (Schwarz and McCarthy, 2008). Accordingly, sex differences might have already developed at an earlier age. Indeed, neonatal boys already have larger total brain, cerebral gray and white matter volumes than neonatal girls (Gilmore et al., 2007). Beside sex hormones, other factors can play a key role as well in establishing an association between regional sex differences and steroid hormones. For example, sex chromosome genes were found to contribute directly to the development of sex differences in the murine brain, independent of gonadal activity (De Vries et al., 2002; Bűdefeld et al., 2008). It might also be argued that brain regions associated with estradiol levels in girls were sexually dimorphic when compared with boys prior to puberty and the change that occurs with increased estradiol levels eliminates this sex difference (De Vries, 2004). This would suggest that a lack of sex differences in these brain areas would persist after puberty despite any future potential changes in male testosterone or estradiol along pubertal development. Also, as males advanced into puberty at a later age and testosterone levels increase, the sex difference might re-emerge. These hypotheses should be addressed in future research.

There are a number of limitations to this study which need to be taken into account when interpreting our findings. Girls in our sample were significantly older than boys, while it was demonstrated earlier that maximum gray matter volume in boys is reached at an older age than in girls (Giedd et al., 1999). Thus, we may have sampled boys during the upward curve of gray matter development and girls during the downward curve. At this stage, it is therefore not possible to dissociate opposing actions of testosterone and estradiol from different hormone actions during different developmental stages. It might also be that at an earlier age, estradiol in females is related to gray matter increases. A further (related) limitation is that in the analyses on sex differences in the brain we linearly corrected for age, but in longitudinal studies nonlinear age-related trajectories have been reported for global and focal brain areas (Giedd et al., 1999; Lenroot et al., 2007). However, since our sample had a substantially smaller age range than the studies by Lenroot et al. (2007) and Giedd et al. (1997, 1999) (10—15 years versus 3—27 and 4—18 years), our applied age-correction may have been appropriate.

Recently, brain morphological changes were reported across the menstrual phase (Protopopescu et al., 2008). To investigate possible confounding effects of intra-individual variation of estradiol levels in the five regularly and three irregularly menstrual cycling girls, we repeated the analyses excluding these eight subjects. Results showed no substantial change of the data: gray matter volume decreases remained significantly related to higher levels of estradiol and the same regional gray matter areas were significantly related to estradiol levels.

A limitation regarding our applied technique: as gonadal hormones likely affect the brain in very subtle ways (i.e. on a cellular level as can be measured with post-mortem and/or animal studies), 1.5-T MRI scans with 1 mm $\times$ 1 mm $\times$ 1.2 mm-voxels in general might not be sensitive enough to measure subtle brain changes due to hormonal development. Also, the relatively small sample size could have caused a lack of power to demonstrate any associations between testosterone and brain structure in either girls or boys.

In conclusion, in girls, with the progression of puberty, gray matter development is at least in part directly associated with increased levels of estradiol, whereas in boys, who are in a less advanced pubertal stage, such steroid-related development could not (yet) be observed. We suggest that in pubertal girls, estradiol may be implicated in the neural remodeling of heteromodal association areas in the cerebral cortex during this important period of brain development.

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NWO had no further role in the study design, in data collection, analysis and interpretation of the data.

Conflict of interest

None declared.

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