Genetic & Environmental risk factors for Obsessive-Compulsive Symptoms: Do they affect the same brain?

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8 Sex differences in gray and white matter structure: converging evidence from complementary methods

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Abstract

Imaging studies on sex differences in human brain structure have reported inconsistent findings. This might relate to suboptimal matching for age, family environment or family background. In addition, previous studies generally focussed on a single structural measure which provides an incomplete picture of male-female brain differences. We investigated sex differences in regional gray matter volume, white matter volume, white matter integrity and cortical thickness in 69 carefully matched male-female pairs. Our results, which we confirmed in a unique subsample of 24 opposite sex twin pairs that optimally controls for genetic, intrauterine and familial environmental factors, provide a comprehensive and robust picture of sex differences in brain structure. Males showed larger gray matter volume and higher fractional anisotropy in, or surrounding, subcortical structures (hypothalamus, putamen, globus pallidus, thalamus) that have been linked to neuropsychiatric disorders with higher prevalence in males, and females had larger gray matter volume and a thicker cortex in the insula and anterior cingulate which have been related to neuropsychiatric disorders with higher prevalence in females. From this, we conclude that sex differences should be considered when studying the neurobiology of neuropsychiatric disorders that differ in prevalence or symptoms between the sexes.

Introduction

Sex differences in human brain anatomy are thought to play a crucial role in the differential sensitivity to psychiatric disorders between males and females (Abel et al., 2010; Parker and Brotchie, 2010; Rucklidge, 2010; Bekker and van Mens-Verhulst, 2007) as well as in sex differences in specific cognitive abilities (Halpern, 1997; Loring-Meier and Halpern, 1999; Mann et al., 1990; Burgaleta et al., 2011). The brains of males and females already begin to differ in an early developmental stage through the action of sex specific factors, such as hormonal, genetic and epigenetic factors (McCarthy and Arnold, 2011), and sex-specific maturation further continues during puberty and adolescence (Sisk and Zehr, 2005). Post-mortem and in vivo imaging studies of both children and adults consistently report that males have an approximately 9-12% larger brain volume than females. Apart from this global volume difference, regional sexual dimorphisms have also been found, primarily for areas with high numbers of sex steroid receptors. After correcting for total brain volume, males tend to have larger gray matter volumes in amygdala and hypothalamus, whereas females tend to have larger orbitofrontal, hippocampal and caudate volumes, for a review see Cosgrove et al. (2007) and Lenroot and Giedd, (2010). However, no difference in hippocampal and amygdalar volumes between males and females (Gur et al.,

2002) or larger hippocampal volume in males compared to females has also been reported (Good et al., 2001). Neuroimaging studies that investigated sex differences in cortical morphometry observed a thicker cortex in males in regions of the temporal lobe (Luders et al., 2006; Sowell et al., 2007; Lv et al., 2010), whereas in females a thicker cortex was observed for frontal (Im et al., 2006; Luders et al., 2004; Sowell et al., 2007; Lv et al., 2010), parietal (Im et al., 2006; Luders et al., 2004; Sowell et al., 2007; Lv et al., 2010) and occipital regions (Im et al., 2006; Luders et al., 2004; Lv et al., 2010). However, there are also studies that found no differences in cortical thickness between males and females (Salat et al., 2004; O'Donnell et al., 2005; Nopoulos et al., 2000; Crespo-Facorro et al., 2011).

Mixed results have also been reported for sex differences in regional white matter volumes. The corpus callosum has been found to be larger in females (Lacoste-Utamsing and Holloway, 1982), larger in males (Sullivan et al., 2001), or of similar size for males and females (Bishop and Wahlsten, 1997). Other regions reported to show significant sex differences include the anterior temporal lobe and internal capsule which was found to be larger in males and the posterior frontal lobe and optic radiation which was found to be larger in females (Good et al., 2001).

To examine sex differences in white matter microstructure in more depth, recent studies have applied diffusion tensor imaging (DTI). The most reported metric derived from DTI is fractional anisotropy (FA), which is a relative measure of the degree of water diffusion anisotropy within a voxel (Mori and Zhang, 2006; Beaulieu, 2002; Basser and Pierpaoli, 1996; Le Bihan et al., 2001) and can be interpreted as a proxy measure of white matter integrity. Higher fractional anisotropy in males has been reported for the internal capsule, thalamus, cingulate, occipito-parietal and temporal regions (Schmithorst et al., 2008; Chou et al., 2011; Menzler et al., 2011), whereas in females, higher fractional anisotropy has been found in the fronto-occipital fasciculus and parahippocampal regions (Chou et al., 2011). For other regions there are again inconsistencies. Higher fractional anisotropy in the left frontal lobe of females has been found (Szeszko et al., 2003), while other studies reported higher frontal fractional anisotropy in males (Schmithorst et al., 2008; Chou et al., 2011). Fractional anisotropy in the corpus callosum was found to be higher in males in some studies (Shin et al., 2005; Menzler et al., 2011) whereas others report higher fractional anisotropy in the corpus callosum of females (Chou et al., 2011; Schmithorst et al., 2008).

Inconsistencies across studies that investigated structural brain differences between the sexes may originate from differences in the analysis technique used (e.g., voxel-by-voxel comparison of fractional anisotropy versus tract-based spatial statistics (TBSS)). Previous studies also generally focussed on a single anatomical

parameter obtained from a specific measurement technique (e.g., gray matter volume, gray matter thickness, white matter volume or white matter integrity). The use of complementary techniques and converging evidence from anatomical measures (e.g., voxel-based morphometry (VBM) and cortical thickness for gray matter structure) would strongly improve the robustness of the findings. In addition and perhaps more importantly, previous studies generally lack a careful matching for age, family environment and family background which have shown to contribute substantially to individual differences in global as well as regional brain volume measures (Thompson et al., 2001; Toga and Thompson, 2005).

The present study aims to study sex differences in structural brain measures more thoroughly, by simultaneously investigating differences in regional gray matter volume, white matter volume, white matter integrity and cortical thickness in 69 carefully matched male-female pairs, including 24 opposite sex twin pairs and 5 male-female sibling pairs close in age. In addition, these four brain measures were re-analyzed in the opposite sex twin pairs only, who are matched not only for age, but also for their early developmental environment, including the intrauterine environment, and part of their genetic background.

Based on previous findings we expect to find larger hypothalamic gray and internal capsule and temporal white matter volumes in males and larger caudate gray matter and frontal gray and white matter volumes in females. Regional sex differences detected by voxel-based mapping of gray and white matter volumes are expected to converge with sex differences in cortical thickness and white matter integrity. These sex differences should be confirmed when controlling for genetic and familial environmental factors.

Methods

Participants

Participants were recruited from an ongoing study in the Netherlands Twin Register (NTR) that investigates environmental and genetic influences on obsessive-compulsive (OC) symptoms (den Braber et al., 2010). We selected unrelated male-female pairs closely matched for age as well as Dizygotic-Opposite-Sex (DOS) twin pairs and male-female sibling pairs (maximum age difference 5 years) in the age range between 18 and 60 years. The DOS twin pairs and sibling pairs are very well matched, not only for age, but also for their early developmental environment and part of their genetic make-up. Exclusion criteria were brain damage, neurological disease, color blindness and contraindications for MRI (e.g., pregnancy, ferromagnetic fragments, clips and devices in the body and claustrophobia). In total, 69 male-female pairs (mean age 30.9, sd=0.71) participated

Table 8.1. Sample characteristics

	Males (*n* = 69)	Females (*n* = 69)	df	t-value	*p* -value
Demography					
Age	31.76±7.64	31.96±7.46	45	-.719	.476
Educational attainment (% low/middle/high)	10.1/30.4/59.4	8.7/34.8/56.5	2	.327*	.849
Global brain measures					
Gray Matter	750.49±61.69	662.47±55.65	68	11.373	<.001
White Matter	529.52±46.40	468.27±43.22	68	10.773	<.001
Total Intracranial Volume	1540.91±125.50	1355.83±114.34	68	11.935	<.001
Mean Fractional Anisotropy	0.30±0.01	0.29±0.01	68	2.791	.007
Mean Cortical Thickness	4.56±0.17	4.59±0.19	68	-1.084	.282

*Age: mean (±SD) age at time of MRI examination (in years); educational attainment (% low/middle/high): percentage of males and females with low, middle or high educational level. df: degrees of freedom. * tested using Chi-square statistics.*

in our MRI study, including 24 DOS twin pairs, 5 sibling pairs and 40 age-matched male-female pairs **(table 8.1)**.

Protocol

Participants were administered diagnostic interviews and questionnaires, including questions on demography, life-events, and neuropsychiatric illness as described elsewhere (den Braber et al., 2010). Educational attainment was assessed as the highest level of education of the participant, divided into 3 categories: 1) lower general and vocational education; 2) intermediate vocational and intermediate/higher general education; 3) higher vocational college and university. The ethical review board of the VU University medical centre approved the study protocol. All participants provided written informed consent.

Image acquisition

The MRI session consisted of an anatomical scan of about 6 minutes and a DTI scan of approximately 3 minutes. During the scan sessions, the participants remained inside the scanner and were asked to minimize head movement during and between consecutive runs. To reduce motion artifacts, each participant's head was immobilized using foam pads.

MRI was performed on a 3.0 T Intera MR system (Philips, Medical Systems, Best) with a standard SENSE receiver head coil. The anatomical scan consisted of 182 coronal slices with a 3D T1-weighted gradient-echo sequence (flip angle 8°; Repetition Time, TR = 9.69 ms; Echo Time, TE = 4.60 ms; matrix, 256x256 pixels;

voxel size, 1.00x1.00x1.20 mm). Diffusion tensor images were obtained in 32 directions by using single-shot echoplanar acquisition (flip angle 90°; Repetition Time, TR = 4834 ms; Echo Time, TE = 94 ms; matrix, 112x110 pixels; voxel size, 2.00x2.00x3.00 mm; b-value 1000 s/mm^2, 38 axial slices).

Data analysis

Regional gray matter and white matter volume differences between males and females were analyzed using VBM as implemented in SPM8 (Wellcome Department of Imaging Neuroscience, London, UK). T1-weighted MR images were segmented into gray matter, white matter and cerebrospinal fluid (CSF), and normalized to a group template (i.e., a specific template created from the 138 subjects that participated in this study) using the Diffeomorphic Anatomical Registration Through Exponential Lie algebra (DARTEL) algorithm, and subsequently warped from DARTEL space to the standard Montreal Neurological Institute (MNI) brain. To preserve volumetric information, a modulation step was added. Before statistical analysis, the resultant modulated images were spatially smoothed with an 8 mm isotropic Gaussian kernel.

To investigate sex differences in white matter integrity, fractional anisotropy maps were calculated from raw DTI scans using the Medical Image Navigation and Research Tool by INRIA (MEDINRIA, Asclepios Research Project - INRIA Sophia Antipolis, France). Fractional anisotropy maps were then co-registered with T1-weighted MR images and normalized using each subject's T1 to DARTEL to MNI warp parameters. Subsequently, data were spatially smoothed with an 8 mm isotropic Gaussian kernel and a voxel-by-voxel comparison of the fractional anisotropy values was performed in SPM8. As an alternative method statistical analysis of the fractional anisotropy data was carried out using TBSS (Smith et al., 2006), part of FSL (Smith et al., 2004), which projects all subjects' fractional anisotropy data onto a mean fractional anisotropy tract skeleton, before applying voxelwise cross-subject statistics. This was done in order to confirm the obtained voxel-by-voxel fractional anisotropy comparison and gives the opportunity to visualize WM differences on a true anatomical tract basis.

To investigate sex differences in cortical thickness delineation of gray and white matter surfaces were determined from MRI using FreeSurfer (Fischl and Dale, 2000) (http://surfer.nmr.mgh.harvard.edu/). Segmentation/boundary tessellation was checked for each scan by means of visual inspection, and manually adjusted when necessary. Subsequently, each individual's surface was registered onto the average surface provided by Freesurfer and spatially smoothed by 5 mm FWHM. Cortical thickness was calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface.

Statistical tests

Sex differences in demographic and global brain measures were tested using paired sample t-tests or Chi-square test (SPSS, Chicago, Illinois). Statistical results were considered significant at $p < 0.05$, Bonferroni corrected.

Differences in regional gray matter volume, regional white matter volume, fractional anisotropy maps, and cortical thickness between males and females were investigated using paired sample t-tests. Group differences are reported at an individual voxel threshold of $p < 0.05$, corrected for multiple comparisons. In addition, a paired sample t-test was performed on the DOS twin pairs only. For the confirmative analysis in this smaller subsample, group differences are reported at an individual voxel threshold of $p < 0.005$, uncorrected.

Results

Sample characteristics

Sample characteristics for the scanned males and females are summarized in the top of **table 8.1**. As expected, matched male-female pairs (excluding DOS twin pairs that are always identical for age) did not differ significantly in mean age ($t(45)=-.719$, $p=<.476$). Furthermore, males and females did not differ with respect to educational attainment ($\chi^2(2)=3.27$, $p=.849$).

Sex differences in global brain measures

Means, standard deviations and t-statistics for global brain volume measures are presented in the bottom of **table 8.1**. Males had larger total intracranial volumes (TIV), gray matter (GM) and white matter (WM). In addition, mean fractional anisotropy was higher in males. No significant differences in mean cortical thickness between the sexes was observed.

Sex differences in regional gray matter

Regional volumes (VBM: adjusted for total intracranial volume)

Table 8.2 (top) summarizes GM regions that were found to be larger in males compared to females, analyzed across all 69 pairs (left) and the DOS pairs only (right). In both analyses, these included 4 subcortical structures: hypothalamus, putamen, globus pallidus and thalamus pulvinar **(figure 8.1, top)**. Furthermore, clusters of larger cortical GM volume were found in the right precentral gyrus, right caudal middle frontal gyrus, left superior temporal gyrus and right cerebellum. From these, only the significant difference for the right precentral gyrus could be confirmed in the DOS sample.

Table 8.2. Sex Differences in Regional Gray Matter Volume

Test	Anatomical location	All pairs (69 males/69 females)				DOS pairs only (24 males/24 females)			
		MNI coordinates			T-value	MNI coordinates			T-value
		x	y	z		x	y	z	
males>females	right precentral	13.5	-30	64.5	3.90	15	-28.5	63	2.83
		24	-10.5	58.5	3.75	--	--	--	--
	right caudal middle frontal	34.5	18	49.5	3.38	--	--	--	--
	right hypothalamus	6	1.5	-12	8.52	6	1.5	-12	3.36
	left hypothalamus	-13.5	1.5	-16.5	5.60	-13.5	-1.5	-15	2.87
	right putamen	24	1.5	7.5	4.71	24	0	7.5	2.93
	left putamen	-24	1.5	9	5.07	-24	1.5	10.5	2.87
	right thalamus pulvinar	15	-33	7.5	4.57	--	--	--	--
	left thalamus pulvinar	-16.5	-34.5	3	4.45	-19.5	-34.5	6	2.89
	right globus pallidus	25.5	-19.5	-4.5	3.89	25.5	-18	1.5	2.89
	left superior temporal	-52.5	21	-13.5	4.73	--	--	--	--
	right cerebellum	49.5	-66	-52.5	3.87	--	--	--	--
females>males	right postcentral	40.5	-19.5	34.5	4.41	--	--	--	--
		22.5	-43.5	48	4.40	--	--	--	--
	left caudal anterior cingulate	-9	3	33	4.81	-10.5	-7.5	34.5	2.93
	right rostral middle frontal	43.5	36	16.5	4.50	46.5	34.5	13.5	2.83
	left insula	-31.5	-28.5	12	4.76	-31.5	-28.5	12	4.84
	left inferior temporal	-48	-25.5	-16.5	4.70	-48	-25.5	-16.5	3.43

Clusters with regional GM differences between males and females, analyzed across all 69 male-female pairs (left), and in the 24 DOS pairs only (right). Test: test for significant GM increases in males compared to females (males > females) or significant GM increases in females compared to males (females > males); Anatomical location: enlarged brain region; MNI coordinates (mm): location of voxel with largest effect size; T-value: t-statistics of voxel with largest effect size. Shaded rows indicate regions that showed both larger GM volume and thicker cortex.

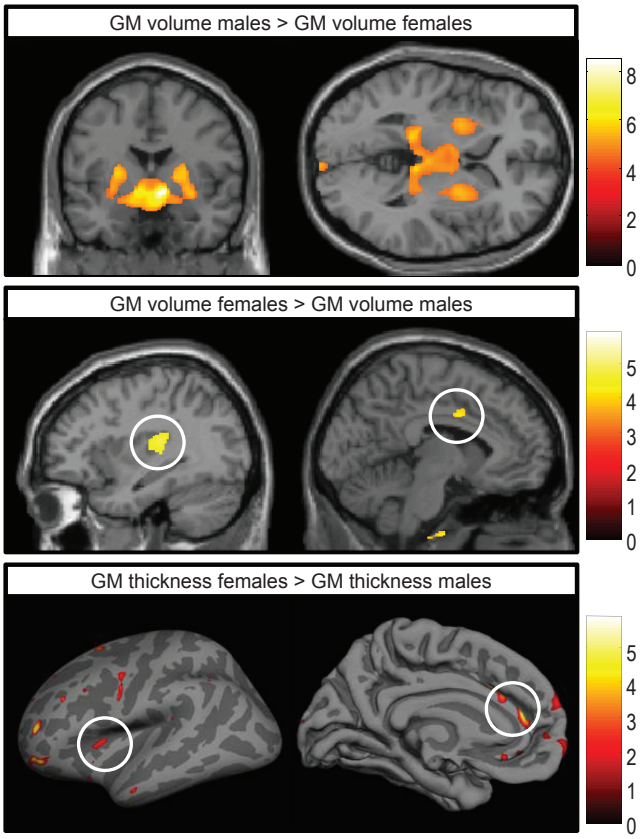

Figure 8.1. *Top: Subcortical brain regions showing larger gray matter volumes in males compared to females (including bilateral hypothalamus, putamen and thalamus pulvinar).*
Middle and bottom: Examples of brain regions showing both larger GM volume as well as higher cortical thickness in females compared to males (left middle encircled: left insular volume enlargement (lateral view); left bottom encircled: thicker left insular cortex (lateral inflated view, gyri in white, sulci in gray); right middle encircled: left anterior cingulate enlargement (medial view); right bottom encircled: thicker left anterior cingulate cortex (medial view, gyri in white, sulci in gray).

The bottom of **table 8.2** shows GM regions that are larger in females. These included the left caudal anterior cingulate gyrus, right rostral middle frontal gyrus, left insula and left inferior temporal gyrus. Two clusters of larger cortical GM were found in the right postcentral gyrus that could not be confirmed in the DOS pairs.

Cortical thickness (Freesurfer)

Table 8.3 (top) summarizes parts of the brain where males showed to have a thicker cortex compared to females, analyzed across all 69 pairs and for the DOS pairs only. In general, for both the left and right hemisphere these included more posterior structures of the brain, such as isthmus cingulate, inferior parietal cortex, precuneus, and lingual cortex, of which the latter two were confirmed in the DOS

pair analysis. For the left hemisphere, additional clusters were observed in the medial orbitofrontal cortex, parahippocampal cortex, middle temporal cortex and temporal pole, from which the latter one could be confirmed in the DOS pairs. For the right hemisphere, additional clusters were observed in the postcentral cortex, supramarginal cortex and lateral occipital cortex, from which the supramarginal cortex and lateral occipital cortex could be confirmed in the DOS pairs.

The bottom of **table 8.3** shows parts of the brain where females were found to have a thicker cortex. In general, for both the left and right hemisphere significant clusters were located in more anterior structures of the brain, such as the parahippocampal cortex, superior frontal cortex, caudal middle frontal cortex, parsorbitalis, lateral and medial orbitofrontal cortex, frontal pole, rostral and caudal anterior cingulate, insula, superior temporal cortex, and temporal pole. All of these were confirmed in the DOS pair analysis, except for the parahippocampal and medial orbitofrontal cortex. For the left hemisphere, additional clusters were observed in the lateral occipital cortex, rostral middle frontal cortex, precentral cortex, middle and inferior temporal cortex, fusiform cortex, and supramarginal cortex, which, except for the lateral occipital cortex, could be confirmed in the DOS pair analysis. For the right hemisphere, additional clusters were observed in the parsopercularis, postcentral cortex, isthmus cingulate, lingual cortex and inferior parietal cortex, from which the postcentral and inferior parietal cortex could be confirmed in the DOS pair analysis.

Interestingly, within some brain regions, including the right postcentral gyrus, left caudal anterior cingulate gyrus, left insula and left inferior temporal gyrus, females showed significant clusters of increased GM volumes as well as increased cortical thickness (middle and bottom row of **figure 8.1** and **table 8.2** and **8.3**, shaded rows).

Sex differences in regional white matter

Regional volumes (VBM: adjusted for total intracranial volume)

The male-female comparison for regional white matter volume revealed no significant sex differences.

White matter integrity – fractional anisotropy (voxel-based comparison/TBSS)

Table 8.4 (top) summarizes clusters of significant higher fractional anisotropy in males compared to females, analyzed across all 69 pairs (left) and for the DOS pairs only (right). In both analyses, significant clusters were observed in the superior longitudinal fasciculus, inferior longitudinal fasciculus, internal and external capsule, anterior thalamic radiation, corona radiata and corpus callosum. These results were confirmed by the analyses of fractional anisotropy using

Table 8.3. Sex Differences in Cortical Thickness

		Left hemisphere								Right hemisphere							
		All pairs (69M / 69F)				DOS pairs only (24M / 24F)				All pairs (69M / 69F)				DOS pairs only (24M / 24F)			
Test	Anatomical label	MNI coordinates			T-value	MNI coordinates			T-value	MNI coordinates			T-value	MNI coordinates			T-value
		x	y	z		x	y	z		x	y	z		x	y	z	
males>females	medial orbitofrontal	-10.4	7.7	-14.9	5.67												
	isthmus cingulate	-5.7	-42.7	30.7	3.78					15.5	-51.0	6.5	3.69				
	postcentral									44.2	-24.8	40.0	4.15				
	parahippocampal	-20.1	-18.5	-26.3	3.48												
	lingual	-22.9	-54.8	0.0	4.16	-19.9	-64.1	-8.9	3.46	4.7	-53.2	3.8	5.50	19.5	-68.1	2.7	3.18
	temporal pole	-32.7	4.1	-14.1	4.78	-32.5	2.5	-14.5	4.27								
	middle temporal	-62.2	-26.0	-13.3	5.20												
	precuneus	-14.7	-49.0	39.1	3.90	-14.5	-49.7	39.1	4.08	5.6	-64.2	28.6	3.72	6.8	-55.7	18.9	2.98
	supramarginal									46.7	-33.7	28.7	3.72	37.2	-36.9	37.0	3.31
	inferior parietal	-38.9	-54.9	14.8	3.43					49.1	-50.2	25.6	4.13	48.2	-47.7	22.2	2.97
	lateral occipital									43.0	-67.0	5.5	4.30	42.5	-67.8	4.8	3.24
females>males	superior frontal	-12.1	54.6	25.2	4.91	-14.8	57.5	15.3	3.19	15.6	37.2	44.0	4.00	16.6	36.1	44.9	4.49
	rostral middle frontal	-36.2	48.9	12.3	5.34	-36.2	44.5	15.3	3.43								
	caudal middle frontal	-40.0	18.1	33.7	3.65					38.5	4.2	45.4	3.67	33.2	6.8	32.1	3.39
	parsorbitalis	-37.8	46.5	-9.7	5.20	-43.7	40.0	-10.9	3.40	43.9	36.9	-12.8	3.77	42.2	43.5	-7.7	2.97
	lateral orbitofrontal	-21.2	18.7	-18.0	4.12					16.9	30.6	-22.6	5.06	24.0	15.0	-18.5	4.37
	medial orbitofrontal	-7.2	28.0	-10.6	3.88					11.6	48.1	-3.4	3.94				
	frontal pole	-11.9	62.5	-5.7	5.65	-12.7	62.5	-5.1	3.75	10.5	64.4	-3.3	3.94				
	parsopercularis									53.0	24.0	15.7	4.01				
	precentral	-55.0	-2.1	36.5	4.27	-57.8	-4.8	11	3.26								
	postcentral									43.0	-28.7	62.1	3.78	48.8	-21.8	55.8	3.02
	rostral anterior cingulate	-7.8	37.3	12.6	5.89	-9.0	34.5	16.5	3.54	7.8	37.0	0.3	3.93				
	caudal anterior cingulate	-7.6	24.7	25.2	4.51	-9.0	16.8	30.7	3.47	7.8	33.8	17.5	4.52				
	isthmus cingulate									9.1	-39.3	26.3	3.70				
	insula	-30.4	10.3	11.7	4.12					32.9	13.1	-7.5	4.24	36.3	-2.0	-7.1	2.91
	superior temporal	-62.8	-26.8	5.1	3.47	-58.1	-6.7	-1.3	4.25	63.6	-36.6	12.7	4.58	50.7	2.5	-21.7	3.20
	middle temporal	-57.3	-5.8	-26.6	4.17	-57.3	-7.3	-24.4	3.29								
	inferior temporal	-45.5	-14.1	-35.8	3.59	-54.4	-26.7	-28.3	3.10								
	temporal pole	-34.6	14.4	-35.3	3.47	-33.6	13.8	-35.3	3.14	37.7	14.9	-35.4	3.87	40.7	13.1	-35.6	4.12
	lingual									14.8	-91.3	-6.8	3.79				
	parahippocampal	-28.1	-20.9	-28.4	3.80					29.5	-30.8	-18.1	3.67				
	fusiform	-33.5	-6.2	-34.0	3.76	-33.6	-37.3	-20.3	3.09								
	supramarginal	-49.3	-54.7	42.2	3.92	-49.1	-54.9	42.2	3.13								
	inferior parietal									40.3	-78.7	17.1	4.22	36.1	-75.9	35.2	4.32
	lateral occipital	-11.2	-101.2	6.8	3.64												

Clusters with cortical thickness differences between males and females for the left and right hemisphere, again analyzed across all 69 male-female pairs and in the DOS twin pairs only. Test: test for brain regions showing significant higher cortical thickness in males compared to females (males > females) or significant higher cortical thickness in females compared to males (females > males); Anatomical label: vertex label as provided by Freesurfer; MNI coordinates (mm): location of vertex with largest effect size; T-value: t-statistics of vertex with largest effect size. Shaded rows indicate regions that showed both thicker cortex and larger gray matter volume.

Table 8.4. Sex Differences in Fractional Anisotropy

		All pairs (69 males / 69 females)				DOS pairs only (24 males / 24 females)			
Test	White matter tract	MNI coordinates			T-value	MNI coordinates			T-value
		x	y	z		x	y	z	
males > females	left superior longitudinal fasciculus	-33	10.5	22.5	4.60	-31.5	13.5	25.5	3.22
	right superior longitudinal fasciculus	39	1.5	28.5	4.27				
	left inferior longitudinal fasciculus	-46.5	-24	-15	3.62	-48	-30	-15	3.51
	right inferior longitudinal fasciculus	49	-13	-4	4.63	52.5	-7.5	-4.5	3.72
	left internal capsule	-21	3	16	4.66	-24	6	15	2.87
	right internal capsule	21	3	16	4.20	24	0	15	2.99
	left external capsule	-30	2	15	4.65	-28.5	4.5	15	3.07
	right external capsule	30.5	-3	14	4.60	30	0	13.5	3.68
	left anterior thalamic radiation	-15	-13.5	13.5	4.15	-15	-33	7.5	3.82
	right anterior thalamic radiation	12	-22	12	3.64	10.5	-15	7.5	2.92
	left corona radiata	-24	12	15	3.01	-25.5	10.5	15	2.90
	right corona radiata	27	12	15	2.60				
	corpus callosum	1.5	16.5	21	4.72	18	27	18	2.94
females > males	left forceps minor	-12	39	-10.5	3.56	-12	42	-3	2.87
	right forceps minor	7.5	34.5	-12	3.21				
	right superior corona radiata	22.5	-15	34.5	3.32	28.5	-6	28.5	2.98
	left corticospinal tract	-4.5	-21	-28.5	3.74	-4.5	-21	-28.5	2.95
	right corticospinal tract	7.5	-19.5	-27	3.22	3	-19.5	-25.5	2.89
	right superior longitudinal fasciculus	45	-6	43	3.92				
	left inferior longitudinal fasciculus	-31.5	4.5	-34.5	4.00	-33	4.5	-31.5	3.01

Clusters with regional fractional anisotropy differences between males and females obtained from voxel-based analysis, measured across all 69 male-female pairs (left), and in the 24 DOS pairs only (right). Test: test for significant fractional anisotropy increases in males compared to females (males > females) or significant fractional anisotropy increases in females compared to males (females > males); White matter tract: white matter tract showing increased fractional anisotropy; MNI coordinates (mm): location of voxel with largest effect size; T-value: t-statistics of voxel with largest effect size.

TBSS (**figure 8.2**).

The bottom of **table 8.4** shows clusters of significant higher fractional anisotropy in females. Significant clusters were observed bilaterally in the forceps minor and corticospinal tract and in right superior corona radiata, right superior longitudinal fasciculus and left inferior longitudinal fasciculus. Most of these findings could be confirmed in the DOS pair analysis, except for the right forceps minor and right superior longitudinal fasciculus. Re-analyses of the fractional anisotropy data using TBSS provided no significant results.

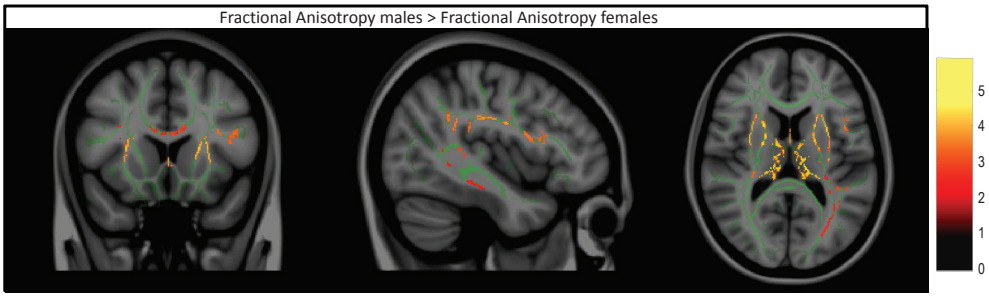

Figure 8.2. *Brain white matter tracts showing increased fractional anisotropy in males compared to females (in red/yellow) projected on a mean fractional anisotropy skeleton (in green) which represents the centers of all tracts common to the group (carried out using TBSS, part of FSL).*

Discussion

The present study aimed to create a more comprehensive picture of sex differences in structural brain measures, by investigating differences in regional gray and white matter volume, white matter integrity and cortical thickness in 69 carefully matched male-female pairs, including 5 sibling pairs and 24 opposite sex twin pairs. In addition, data were re-analyzed in 24 opposite sex twin pairs only, in order to measure whether perfectly controlling for genetic, intrauterine and familial environmental factors would affect the results. Our analyses indicated that males have larger gray matter volumes and higher fractional anisotropy in, or close to, subcortical brain regions (e.g., hypothalamus, thalamus, putamen), whereas females have both larger gray matter volumes as well as greater cortical thickness in insular, anterior cingulate, postcentral and inferior temporal cortices. Results are discussed in more detail below, with a specific focus on regions that held in the opposite sex twin pair analysis and/or brain regions in which sex differences were confirmed by different structural brain measures (gray/white matter volume, fractional anisotropy, cortical thickness).

Sex differences in global brain measures

This study showed that males have significantly larger total intracranial volumes and a higher fractional anisotropy across the brain. The finding of larger global brain volumes in males is in line with the previous literature and is one of the most robust findings on sex differences in the brain, for review see Cosgrove et al. (2007) and Lenroot and Giedd, (2010). Higher mean fractional anisotropy in males has also been found previously (Kang et al., 2011). It has been reported that male brains possess higher neuronal densities, a higher number of neurons and fewer neuropil (i.e., neuronal and glial processes) (de Courten-Myers, 1999), with greater white matter volume available for the inter-neuronal connections (Gur et al., 1999; Allen et al., 2003). From this, one might expect males to have fewer but thicker, more organized, and possibly more myelinated fibers, which would explain the higher mean fractional anisotropy observed in males. No sex differences with regard to mean cortical thickness were found, which replicates previous findings (Im et al., 2006; Salat et al., 2004; Crespo-Facorro et al., 2011).

Sex differences in regional gray matter

Males showed more gray matter primarily in subcortical brain regions, such as hypothalamus, thalamus, putamen and globus pallidus, and higher cortical thickness in temporal regions (lingual gyrus and temporal pole) and parietal regions (inferior parietal and precuneus). Females, on the other hand, mainly had more gray matter and a thicker cortex in insular, anterior cingulate, postcentral and inferior temporal brain areas. A thicker cortex was also observed in frontal brain regions (e.g., superior frontal, rostral/caudal middle frontal, orbitofrontal). Interestingly, these regions contain high levels of androgen and estrogen steroid receptors (Goldstein et al., 2001) and therefore are more likely to exhibit sexual dimorphisms.

Our finding of a larger hypothalamus in males is consistent with previous studies (Goldstein et al., 2001; Bao and Swaab, 2010). This brain region contains significant populations of sex steroid receptors, plays a central role in the control of sexual and reproductive function, has been related to sexual orientation and plays a major role in sexual arousal and psychosexual identity (the personal self-representation of being a 'male' individual) (Swaab et al., 2002; Brunetti et al., 2008).

Together with the caudate nucleus, the putamen is regarded the main receptive component of the basal ganglia. Anatomically it is connected to the frontal cortex through a series of basal ganglia-thalamocortical loops that all run via the globus pallidus (Alexander et al., 1986; Haber, 2003). Together with the thalamus and supplementary motor areas the putamen and globus pallidus are principally involved in the so-called motor loop that plays an important role in the

programming and control of movement. Larger volumes of these regions, or higher neural densities have also been found by others (Giedd et al., 1997; Peper et al., 2009a). Interestingly, abnormalities within these regions have been linked to neuropsychiatric disorders that show a higher prevalence in males compared to females, such as tic disorders and schizophrenia (Shenton et al., 2001; Singer and Minzer, 2003).

Regional volume enlargements and higher cortical thickness in insular, anterior cingulate, and prefrontal regions in females have also been observed previously, especially in studies that focussed on cortical thickness measures (Good et al., 2001; Goldstein et al., 2001; Im et al., 2006; Luders et al., 2006; Sowell et al., 2007; Lv et al., 2010). These structures play a major role in emotion and interoceptive awareness (the sense of the physiological condition of the body). The insula has been associated with both detection and experiencing disgust (Wicker et al., 2003), whereas the orbitofrontal cortex (found to be thicker in females) is involved in emotional decision making (Bechara et al., 2000). The anterior cingulate, ventromedial prefrontal and lateral prefrontal cortices have been associated with integrating interoceptive information, and the insula has been found to play an important role in interoceptive attention (Critchley et al., 2004). Moreover, the perception of bodily state, and simultaneous activation of anterior cingulate, insular and prefrontal regions, was proposed as a crucial determinant for the processing and subjective experience of feelings (Pollatos et al., 2007). Interestingly, abnormalities within these brain regions have been found in neuropsychiatric disorders that show higher prevalences in females, such as depression and anxiety disorders and especially the latter is highly associated with altered bodily responses, including sweating, increased heart rate and blood pressure.

During a large part of human evolution males and females had different social roles (e.g., males: hunting, protect group from predators, make and use weapons; females: gather and prepare food and clothes, and care for the children). They also differ substantially in their optimal mating behavior (Buss, 2000; Eagly and Wood, 1999). Our finding of larger regional volumes in brain structures that play a central role in the control of sexual and reproductive function in males and in regions involved in more social emotional skills in females fits this evolutionarily perspective.

Sex differences in regional white matter

Our study did not reveal any difference in regional white matter volume. However, males and females did show significant differences in fractional anisotropy. In males higher fractional anisotropy was observed mainly in white matter tracts close to subcortical brain regions, such as internal and external capsule, anterior thalamic radiation, corona radiata, but also in corpus callosum, superior

longitudinal fasciculus and inferior longitudinal fasciculus. Higher fractional anisotropy surrounding subcortical brain regions is in line with previous reports (Chou et al., 2011; Menzler et al., 2011) as is the higher fractional anisotropy in the corpus callosum (Shin et al., 2005; Menzler et al., 2011). Although the latter finding has not been unequivocal (Schmithorst et al., 2008; Chou et al., 2011) we note that in our unique subsample of opposite sex twin pairs, that optimally controls for genetic, intrauterine and familial environmental factors, the higher corpus callosal fractional anisotropy holds for both the voxel-based comparison and TBSS.

Females showed higher fractional anisotropy primarily in frontal and temporal brain regions, including forceps minor and superior corona radiata, which is in line with results of a previous study (Szeszko et al., 2003), although higher fractional anisotropy in frontal regions has also been reported for males (Schmithorst et al., 2008; Chou et al., 2011). Of note, tract-based spatial statistics could not replicate these findings. However, it should be noted that most of the voxel-based comparison results were located near the white to gray matter boundary. Tract-based spatial statistics makes use of a mean fractional anisotropy tract skeleton that represents the centers of all tracts common to the whole group and usually does not include small tracts near this boundary. Fractional anisotropy differences in small white matter tracts near cortical regions could therefore be easily missed using tract-based spatial statistics.

The use of opposite sex twin pairs in the male-female comparison is a considerable strength of this study in terms of optimally controlling for genetic, familial environmental, and intrauterine factors. It has been hypothesized that females with a male co-twin might be exposed to higher testosterone levels than other women and experience a relative masculinization of the brain. Evidence for a larger total brain volume in opposite-sex female twins compared to same-sex female twins was reported in 9-year old children, but was not found in adults (Peper et al., 2009b). Masculinization would make our comparison more conservative, i.e., sex differences would be attenuated. As the sex differences in our opposite sex twin pairs were robust and highly consistent with the sex differences in the overall sample, the advantages of optimal matching in opposite sex twin pairs seem to outweigh the confounding by potential differences in intrauterine testosterone exposure.

In summary, by simultaneously investigating differences in regional gray matter volume, white matter volume, white matter integrity and cortical thickness in 69 carefully matched male-female pairs, and by confirming our findings in a unique subsample of opposite sex twin pairs that optimally controls for genetic, intrauterine and familial environmental factors we were able to create a more comprehensive and robust picture of sex differences in brain structure. Our data

shows males to have larger gray matter volumes and higher fractional anisotropy in, or surrounding, subcortical structures. These brain structures are involved in the control of sexual and reproductive function (hypothalamus) and the programming and control of movement (putamen, globus pallidus, thalamus) and have been associated with neuropsychiatric disorders that show a higher prevalence in males (tic disorders, schizophrenia). Conversely, females were characterized by larger gray matter volumes and greater cortical thickness in brain regions importantly involved in emotion and interoceptive awareness (insula, anterior cingulate) and associated with neuropsychiatric disorders that have a higher prevalence in females (depression, anxiety disorders). The observed sex differences in regional brain structure provide a rich source of information for understanding the behavioral differences that exist between males and females. Sex differences should always be considered in studies on the neurobiology of neuropsychiatric disorders that differ in prevalence or symptoms between the sexes.