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# **VISUO-SPATIAL COGNITION IN GENDER DYSPHORIC GIRLS- ORGANIZATIONAL & ACTIVATIONAL EFFECTS OF TESTOSTERONE**

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# Abstract

Sex differences in performance and in cerebral activation patterns during mental rotation have been reported repeatedly and reflect both organizational and activational effects of sex hormones. In the current prospective functional magnetic resonance imaging study, 21 adolescent girls diagnosed with Gender Dysphoria (GD; DSM-5, American Psychiatric Association 2013) were tested twice: when receiving medication to suppress their endogenous sex hormones, just before the onset of testosterone treatment, and then 10 months later while being on testosterone. Our first aim was to investigate whether girls with GD might have undergone an atypical sexual differentiation of the brain during early development, resulting in a more male-typical brain activation pattern when performing the mental rotation task. Two age-matched control groups

of 20 boys and 21 girls participated twice as well. We replicated the normative sex difference, i.e. control girls showed significantly increased activation in frontal brain areas compared to control boys. Girls with GD, prior to testosterone treatment, differed significantly from the control girls only, suggesting a masculinization of brain structures associated with visuo-spatial cognitive functions. A second goal of the study was to test the effects of 10 months testosterone treatment on visuo-spatial functioning, while controlling for developmental effects. We found that girls with GD showed testosterone-related increases in brain activation in areas implicated in mental rotation. Our findings provide new evidence for the differential organizational and activational effects of testosterone on visuo-spatial cognitive functioning.

**T**HE MENTAL ROTATION TASK (Shepard and Metzler 1971; Vandenberg and Kuse 1978), which is a visuo-spatial cognitive task, has consistently been shown to elicit robust sex differences in performance with males outperforming females (Linn and Petersen 1985; Crucian and Berenbaum 1998). Presumably, men and women use different cognitive strategies when they have to determine whether two differently rotated 3-dimensional figures are identical or form mirror images of each other. Thus, men are supposed to apply a more automatic, bottom-up, or *gestalt* processing



strategy during mental rotation, whereas women are thought to solve this task in a more top-down manner, applying a serial reasoning strategy (Thomsen et al. 2000; Butler et al. 2006). Accordingly, functional magnetic resonance imaging (fMRI) studies found sex differences in brain activation patterns associated with mental rotation. A recent meta-analysis showed that a distinct network of parietal and frontal brain areas is implicated in mental rotation (Zacks 2008). Stronger superior parietal activations during mental rotation have been observed in men, whereas women recruit (inferior) frontal and temporal brain areas more compared to men (Hugdahl et al. 2006).

It is widely accepted that sex differences in visuo-spatial cognition evolve during early development under the organizational influence of sex hormones. Androgens in particular are thought to be responsible for the male superiority in visuo-spatial cognition (Janowsky et al. 1998; Zitzmann 2006).

Evidence for early hormonal effects on later visuo-spatial abilities comes from twin studies, which showed enhanced visuo-spatial working memory performance in young women having a male co-twin, compared to women having a same-sex co-twin (Vuoksima et al. 2010; Heil et al. 2011). In addition, studies in women with congenital adrenal hyperplasia (Mueller et al. 2008; Puts et al. 2008; Berenbaum et al. 2012), and in men with idiopathic hypogonadotropic hypogonadism (Hier and Crowley 1982), two clinical conditions characterized by aberrant androgen production, suggest that androgens affect spatial abilities.

Sex differences in mental rotation performance have already been found in children (Kerns and Berenbaum 1991; Pezaris and Casey 1991; Grimshaw et al. 1995; Vederhus and Krekling 1996; Levine et al. 1999; Manson 2008; Clements-Stephens et al. 2009), suggesting that sex differences in spatial functioning observed in adulthood might reflect sex differences in exposure to pre- and perinatal androgens during the sexual differentiation of the brain. However, some studies failed to observe sex differences in mental rotation functions in children, in contrast to older age groups (Roberts and Bell 2000, 2002; Kucian et al. 2007), which suggests that postnatal factors, such as puberty, cognitive development, and experience may also affect the sex-specific development in mental rotation functioning.

In line with this reasoning, the magnitude of the sex difference in mental rotation has been shown to be greater in adolescents (Cohen's  $d$  (Cohen 1988) effect size = 1.01, Kaufman 2007;  $d$  = .45, Voyer et al. 1995) and adults ( $d$  = .66,



Voyer et al. 1995;  $d = 1.24$ , Titze et al. 2010) than in children ( $d = .33$ , Voyer et al. 1995,  $d = .45$ , Titze et al. 2010).

Significant effects of age and age by sex interactions in studies on mental rotation performance during adolescence (Schweinsburg et al. 2005; Titze et al. 2010) suggested that activational effects of sex hormones, starting at puberty, reinforce the sex differences in visuo-spatial functioning. Indeed, mental rotation performance and related brain activation have repeatedly been shown to vary as a function of circulating androgen levels, as well as other sex hormones, such as progesterone and estrogen (Hausmann et al. 2000; Zitzmann et al. 2001; Hooven et al. 2004; Schöning et al. 2007; Cherrier et al. 2010; Griksiene and Rukšenas 2011; Mendrek et al. 2011; Stangl et al. 2011; Vuoksimaa et al. 2012; Courvoisier et al. 2013).

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Individuals with Gender Dysphoria (GD; DSM-5 (American Psychiatric Association 2013)); also referred to as transsexualism (ICD-10 (World Health Organization 1992)) are characterized by a profound feeling of incongruence between their natal sex and expressed/experienced gender. It has been hypothesized that atypical levels of perinatal sex steroids during a critical period of sexual differentiation of the brain may be involved in the development of GD (van Goozen et al. 2002; Swaab 2007). GD has been applied as a *natural model* in order to study the differential effects of the genetic sex, the perinatal hormone environment, and circulating sex hormones in their contribution to the development of gender differences in cognition and behavior. Studies involving individuals with GD, receiving *cross-sex hormonal (CSH)* treatment (estrogens for natal males and testosterone for natal females) as a first step of sex reassignment, provide important, otherwise unethical, research opportunities on how sex hormones affect brain functions.

Neuropsychological studies involving adult individuals diagnosed with GD have yielded some support for both organizational and activational effects of testosterone on mental rotation performance. Treatment-naïve subjects performed comparable to their experienced gender control groups (e.g. women with GD similar to control men) (Zucker and Bradley 1995; Cohen-Kettenis et al. 1998; van Goozen et al. 2002), and CSH treatment improved performance in natal women and had detrimental effects in natal men (Van Goozen et al. 1994, 1995; Slabbekoorn et al. 1999). However, other studies failed to observe early or later sex hormone dependent changes, or differences in spatial abilities



between individuals with GD and controls (Haraldsen et al. 2003, 2005; Wisniewski et al. 2005).

A small number of fMRI studies (Sommer et al. 2008; Carrillo et al. 2010; Schöning et al. 2010) investigated sex-typical (similar to natal sex) and sex-atypical (similar to experienced gender) brain functioning during mental rotation in treatment-naïve (endogenous hormonal status) individuals with GD, as well as in subjects receiving CSH treatment. Results of these studies indicate that adult natal men with GD, regardless of their hormonal status, differ significantly from control men, by showing less activation in left parietal brain areas (Schöning et al. 2010). Carrillo et al. (2010) found that natal men with GD, while receiving estrogen treatment, showed less brain activation in parietal regions than control men, but increased activation in prefrontal areas compared to control women. The authors concluded that men with GD share some male- as well as female-typical mental rotation task associated brain activations, thereby having an intermediate position between the control groups. Remarkably, no effects were found in women diagnosed with GD (Carrillo et al. 2010). Similarly, Sommer et al. (2008) failed to find any significant differences in brain activation during mental rotation between small groups of eight men and six women diagnosed with GD. However, three months after the start of CSH treatment, mental rotation-associated brain activation was positively correlated with serum testosterone levels, suggesting activational effects of testosterone.

Taken together, previous studies involving adult individuals with GD provide some insight into the effects of androgens on visuo-spatial cognition. However, most reports focused on sex-typical or -atypical cognitive functioning of men with GD and thus on the activational effects of estrogen treatment, whereas the association between testosterone and neuro-imaging correlates of spatial cognition in women with GD remains understudied. In addition, it should be noted that men and women diagnosed with GD differ on important aspects, such as their sexual orientation and age of onset of the GD (more often non-homosexual orientation and late-onset diagnosis in natal men) (Lawrence 2010; Nieder et al. 2011; Zucker et al. 2012; Cerwenka et al. 2014).

Therefore, inconsistent results of the above-mentioned studies may be attributable to the fact that heterogeneous samples of participants with GD were involved (mainly natal men with GD), being variable in terms of sexual orientation and age of onset of their GD diagnosis as well as with regard to the type and dosage of the CSH treatment administered.



In the current prospective study, the first aim therefore was to investigate whether a carefully selected, highly homogeneous (in terms of GD onset age, sex, sexual orientation, dosage and type of the CSH treatment administered) group of 21 adolescent natal girls with GD would show a more male- or female-typical brain activation pattern during an fMRI mental rotation task prior to the start of the testosterone treatment. At the *Center of Expertise on Gender Dysphoria* in Amsterdam, adolescents with persisting GD may start treatment with gonadotropin-releasing hormone analogues (GnRHa) at the age of 12 years to suppress endogenous gonadal stimulation and thus the irreversible development of sex-characteristics of the natal sex. Then, at the age of 16 years, as a first step in the actual sex reassignment, they will receive testosterone treatment (Kreukels and Cohen-Kettenis 2011; de Vries and Cohen-Kettenis 2012).

A second aim of the present study was to investigate the effects of testosterone on mental rotation performance and associated brain functioning. Thus, girls with GD participating in the current study were tested twice: shortly before receiving testosterone treatment while their endogenous sex hormones were suppressed, and then again 10 months later while being on testosterone. We hypothesized that the girls with GD, based on the assumption that they have undergone a more masculinized early neuronal sexual differentiation, would show male-typical mental rotation functions (organizational effects). In addition, we expected to observe a testosterone-dependent improvement in task performance and a more male-typical cerebral activation pattern during mental rotation after the testosterone treatment (activational effects).

## Materials and Methods

### SUBJECTS

TWENTY-ONE ADOLESCENT girls (mean age 16.1 years,  $SD = 0.8$ ), all diagnosed with *early onset* GD were recruited via the *Center of Expertise on Gender Dysphoria* at the VU University Medical Center in Amsterdam. The control subjects, 20 boys (mean age 15.9,  $SD = 0.6$ ) and 21 girls (mean age 16.3 years,  $SD = 1.0$ ), were recruited via several secondary schools in the Netherlands, and by inviting friends of the participants with GD. When scanned for the first time (session 1), girls with GD had been treated monthly with 3.75 mg of Triptorelin (Decapeptyl-CR<sup>®</sup>,



Ferring, Hoofddorp, the Netherlands) by injection for on average 24 months (range 2–48 months) resulting in complete suppression of gonadal hormone production. At scan session 2, girls with GD had been receiving testosterone treatment for on average 10 months (range 6–15 months). All girls with GD either received an ester-testosterone mixture (Sustanon® 250 mg/ml, Merck Sharp & Dohme B.V., Oss, the Netherlands) every 2 weeks ( $N=14$ ) or undecanoate-testosterone (Nebido® 250 mg/ml, Bayer, Mijdrecht, the Netherlands) every 12 weeks ( $N=7$ ). The starting dosage varied with the patient's age. Until the age of 16.5 years, the starting dosage was 25 mg/m<sup>2</sup> body surface area every two weeks. When older than 16.5 years the dosage was 75 mg every two weeks (Hembree et al. 2009).

One control girl and four control boys dropped out of the study after the first session. Thus, 16 control boys, 20 control girls, and all 21 girls with GD participated in session 2. Control subjects were exposed to their endogenous sex hormones during both test sessions. Female controls were tested randomly according to their menstrual cycle and screened for hormonal contraceptive use.

## PROCEDURE

ALL PARTICIPANTS COMPLETED four subtests (arithmetic, vocabulary, picture arrangement, and block design) of the Wechsler Intelligence Scale for Children (Wechsler 2005a) or, if older than 16 years of age, the Wechsler Intelligence Scale for Adults (Wechsler 2005b). Each four-subtest sum score was converted to an individual's estimated *Intelligence Quotient* (IQ) score. Sexual orientation was assessed by asking whether the participant had ever been in love with somebody, and whether that person was a boy or a girl. Handedness was measured with the dimensional Edinburgh handedness inventory (Oldfield 1971). All participants and their legal guardians gave their informed consent according to the Declaration of Helsinki, and the study was approved by the Ethics Committee of the VU University Medical Center Amsterdam (application number NL31283.029.10).

## FMRI MENTAL ROTATION TASK

SUBJECTS WERE PRESENTED WITH Shepard and Metzler type three-dimensional (3D) white drawings on a black background taken from the mental rotation



stimulus library, provided by Peters & Battista (2008). In the mental rotation condition, subjects were presented 40 pairs of 3D shapes, with one shape rotated along the x-plane (half of the presented pairs) or the z-plane, relative to the other shape. Stimuli could be rotated at nine different angles, with at least 80 degrees difference between the two presented shapes. Stimuli were presented using a classical block design, with 16 alternating rotation/control blocks, and each block contained five mental rotation or control trials. During the mental rotation condition, subjects had to indicate (by pressing a button) whether the two shapes were identical or mirror images. During the control condition one of the 3D stimuli was presented next to an arrow pointing either to the left or right. Participants were asked to indicate the side to which the arrow was pointing. Presentation duration of each stimulus was variable, depending on the subject's performance, with a maximum stimulus presentation of 20 seconds. Outcome parameters were the percentage of trials correctly identified, and mean reaction time per trial. Before the scanning session, each participant performed a practice session to ensure participants comprehended the task.

### IMAGING PROTOCOL

ALL SCANS FOR SESSION 1 were performed on a 3.0 Tesla GE Signa HDxt scanner (General Electric, Milwaukee, WI, USA). A gradient-echo echo-planar imaging sequence was used for functional imaging. The parameters included a 24 cm<sup>2</sup> field of view, TR of 2100 ms, TE of 30 ms, an 80° flip angle, isotropic voxels of 3 mm, and 40 slices. Before each imaging session a local high-order shimming technique was used to reduce susceptibility artifacts. For co-registration with the functional images a T1-weighted scan was obtained (3D FSPGR sequence, 25cm<sup>2</sup> field of view, TR of 7.8 ms, TE of 3.0 ms; slice thickness of 1 mm, and 176 slices). During the course of the project, a major scanner upgrade (hardware and software) was performed. Although all settings of the scanning protocol remained unchanged, in order to account for possible effects of the upgrade, we counterbalanced session 2 scans over groups (all session 1 scans were performed before the upgrade). Thus, for all session 2 scans, approximately one half of the participants of each group were tested before the upgrade was carried out and the other half of each group was scanned with the upgraded GE scanner, type MR750.





## NEUROPSYCHOLOGICAL TESTS

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NEXT TO THE MENTAL rotation task accomplished during the fMRI sessions, every participant also completed a computerized version of the original Vandenberg & Kuse mental rotation paper & pencil task (Vandenberg and Kuse 1978) outside the scanner. Again, stimuli were taken from the stimulus battery provided by Peters & Battista (2008). During 16 trials, subjects had to indicate which two out of four 3D figures were rotations of the target stimulus. A maximum score of 32 points (max. 2 points per trial) could be achieved. There was no time limit for completing the task and the task was practiced beforehand. Outcome parameters were the percentage correctly identified stimuli, and mean reaction time per trial.

In addition, a computerized version of the *Judgment of Line Orientation* (JOLo) task (Benton et al. 1978) was used to test visuo-spatial working memory. The JOLo requires participants during 30 trials to identify which two out of 11 lines presented in a semicircular array have the same orientation in a two-dimensional space as two target lines presented previously. The original task was adapted in order to avoid ceiling effects; a working memory component was introduced: the two target lines were shown for one second, and then disappeared, and directly thereafter the 11-line array was presented, asking subjects to match the two target lines held in working memory to two lines from the array. A maximum score of 60 points (max. 2 points per trial) could be achieved. The outcome parameters were the percentage correctly identified lines and the mean reaction time per trial. Only the girls with GD performed the neuropsychological tasks during both test sessions. Therefore, session 2 computer MRT and JOLo task data were not available for the control groups.

## DATA ANALYSES

### BEHAVIORAL DATA

Demographic, self-report, and performance data from the JOLo, and both the computer and the fMRI mental rotation tasks were analyzed using the Statistical Package for the Social Sciences, version 20 (SPSS Inc., Chicago, IL, USA). Differences in group characteristics and performance were analyzed using one-way ANOVA. A repeated measures ANOVA was conducted to assess session effects in fMRI mental rotation task performance, with accuracy and mean



reaction time per trial as within-subject factors and group as between-subject factor, including IQ as a covariate. The significance level was set at  $p < 0.05$ .

#### NEUROIMAGING DATA

fMRI data analysis was performed with SPM8 software (Statistical Parametric Mapping; Wellcome Department of Imaging Neuroscience, Institute of Neurology at the University College London, UK) implemented in Matlab R2012b (Math Works Inc., Natick, MA, USA). Functional images were slice-timed, re-aligned to the mean image, and co-registered with the individual anatomical image. Applying the *New Segment* and *Create Template* options of the DARTEL (*Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra*) toolbox, structural images were segmented. Then, gray matter and white matter images were used for creating a group-specific template registered in Montreal Neurological Institute (MNI) space. Functional images were spatially normalized to the group-template, applying each individual's DARTEL flow field and finally, images were smoothed by means of an 8mm *full width half maximum* (FWHM) isotropic Gaussian kernel. First-level contrast images were built by subtracting control trial blocks from mental rotation blocks. Based on the image realignment process, individual head jerks were identified ( $> 1\text{mm}$  displacement) (Lemieux, Salek-Haddadi, Lund, Laufs, & Carmichael, 2007). Together with the six motion parameters, these so-called scan nulling regressors were included in every first-level design matrix to account for the effects of excessive head motion.

Second-level random effects analyses were conducted, entering all individual contrast images (mental rotation  $>$  control condition) from session 1 into a one-way ANOVA in order to test for sex differences (control boys versus control girls), and whether girls with GD at baseline, thus during hormonal suppression and prior to CSH treatment, compared to the control boys and control girls, showed a female- or male-typical mental rotation activation pattern.

By means of a flexible factorial design, modeling within-group effects of the factor session and group by session interaction effects, we investigated the effects of testosterone treatment (session 2 versus session 1), while controlling for possible cognitive developmental and/or learning effects. Thus, adding both control groups to the design controlled for possible within-subject effects other than the testosterone treatment.



Two whole-brain linear regression analyses in the girls with GD were used to explore regions showing testosterone-induced changes in brain activation associated with mental rotation (mental rotation > control, session 2 > session 1) that varied linearly with a) treatment duration in months ( $M = 9.7$ ;  $SD = 3.0$ ; range 5.5 – 14.8) and b) the cumulative dosage in milligram ( $M = 1428.4$ ;  $SD = 764.7$ ; range 440.0 – 3250.0) of testosterone subjects had received between the two scan sessions.

According to a meta-analysis of neuroimaging studies on brain regions implicated in mental rotation (Zacks 2008), we focused our imaging analyses on predefined *regions of interest* (ROI), encompassing the intra-parietal sulcus, the precentral sulcus and the inferior frontal sulcus. These three bilateral ROIs were selected from the Nielsen & Hansen's volume of interest BrainMap database (Nielsen and Hansen 2002). Using the *Marsbar* tool (Brett et al. 2002), the anatomical ROIs were masked with the control groups' mental rotation task main effect (applying a whole-brain threshold of  $p < 0.05$  family-wise error (FWE) corrected), in order to create four separate ROIs: the precentral and the inferior frontal sulcus combined were defined as *frontal* ROI and the intra-parietal sulcus was defined as *parietal* ROI, for the right and left hemisphere, respectively. All comparisons were co-varied for IQ and effects were considered statistically significant at  $p < 0.05$ , voxel-wise FWE-corrected for the spatial extent of the ROI and a minimum cluster size of 20 voxels.

## Results

### DEMOGRAPHICS & SUBJECT CHARACTERISTICS

DEMOGRAPHIC, SELF-REPORT and subject characteristics are presented in Table 1. The IQ scores of the girls with GD were significantly lower than those of both control groups; therefore, IQ scores were included as a covariate in all further between-group analyses. Eleven out of 21 (session 1), and 15 out of 20 (session 2) control girls reported using hormonal contraception. The groups did not differ with regard to age during either test session and were homogeneous with regard to sexual orientation, i.e. all control boys and girls with GD were gynephilic and all control girls were androphilic.



**Table 1** Demographics and subject characteristics

	SESSION	GIRLS W. GD	CTRL GIRLS	CTRL BOYS	F (DF)	P VALUE
N	1	21	21	20		
	2	21	20	16		
Age (mean (SD))	1	16.1 (0.8)	16.3 (1.0)	15.9 (0.6)	1.5 (2, 60)	.244
	2	17.0 (0.8)	17.6 (0.8)	17.2 (0.7)	2.4 (2, 54)	.101
IQ (mean (SD))		100.5 (12.7)	110.3 (14.7)	113.4 (14.5)	5.1 (2, 59)	<b>.009</b>
Sexual Orientation		100% gynephilic	100% androphilic	100% gynephilic		

Ctrl = control; GD = Gender Dysphoria; IQ = Intelligence Quotient

**Table 2** Performance data visuo-spatial cognitive tasks

	TASK	SESSION	GIRLS W. GD	CTRL GIRLS	CTRL BOYS	F (DF)	P VALUE
% correct	fMRI MRT	1	66.7 (15.9)	67.0 (11.6)	70.2 (10.7)	0.5 (2, 59)	.638
		2	74.2 (9.0)	71.7 (8.2)	71.6 (10.3)	0.5 (2, 54)	.598
<i>Cohen's d</i>			-0.59	-0.48	-0.14		
% correct	MRT	1	78.6 (14.4)	82.8 (12.7)	84.4 (13.9)	1.0 (2, 58)	.379
		2	86.7 (12.4)				
% correct	JoLO	1	69.7 (10.6)	73.0 (9.6)	76.5 (8.0)	2.7 (2, 58)	.078
		2	73.8 (11.1)				
RT/trial	fMRI MRT	1	8.0 (2.2)	8.2 (1.5)	8.1 (1.6)	0.04 (2, 59)	.963
		2	6.7 (2.1)	6.8 (1.7)	7.5 (2.0)	1.0 (2, 54)	.386
<i>Cohen's d</i>			0.62	0.90	0.35		
RT/trial	MRT	1	31.1 (13.1)	32.9 (10.3)	29.9 (12.3)	0.3 (2, 58)	.734
		2	24.6 (10.1)				
RT/trial	JoLO	1	3.4 (0.9)	3.4 (.4)	3.2 (.7)	0.8 (2, 58)	.445
		2	3.5 (0.8)				

Ctrl = control; GD = Gender Dysphoria; fMRI = functional magnetic resonance imaging; MRT = mental rotation task; RT = reaction time; JoLO = Judgment of Line Orientation; Cohen's d = effect sizes are calculated for group means at session 1 versus session 2, using the pooled standard deviation of the two means. Session 2 data for the tasks performed outside the scanner were only available of the girls with GD

## BEHAVIORAL DATA

ONE-WAY ANOVA YIELDED no significant group differences in performance on any of the visuo-spatial cognitive tasks (see Table 2).



The repeated measures ANOVA revealed a significant main effect of session in fMRI mental rotation accuracy ( $F(1, 53) = 11.9, p = .001$ ). No main effect of group or any group by session interaction was observed. Visual inspection of the data suggested a greater improvement in fMRI mental rotation performance for both the girls with GD and the control girls (mainly in reaction times), whereas the performance of the control boys remained stable. This was confirmed by effect size calculations using Cohen's  $d$ , which revealed moderate to strong effects, thus improvement in reaction times and accuracy, in the two natal female groups and only small effect sizes in the males, while correcting for group differences in IQ (see Table 2).

## NEUROIMAGING DATA

### FMRI MENTAL ROTATION TASK MAIN EFFECT

During mental rotation all three groups showed widespread task-related bilateral activations, recruiting parieto-occipital and frontal networks (see Figure 7.1).

FIGURE 7.1 >  
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### GROUP DIFFERENCES IN FMRI MENTAL ROTATION

The between-group comparisons at baseline (session 1), adjusted for group differences in IQ, revealed significant sex differences in mental rotation associated brain activation. Control girls showed several clusters of increased activation compared to control boys in the right frontal and the left parietal ROI. The reverse contrast, testing for any increased activation during mental rotation in control boys over control girls yielded no significant effects. Comparing control girls to girls with GD revealed a significant activation in the right frontal ROI, similar to the sex difference observed between the control groups (see Figure 7.2 and Table 3). None of the other between-group comparisons revealed any significant effects.

FIGURE 7.2 >  
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### TESTOSTERONE-INDUCED EFFECTS

Two of the contrasts testing group by session interactions, control boys > control girls and girls with GD > control girls, revealed significant effects in the left frontal and both parietal ROIs (see Table 4). No other significant interaction effects were found. Post-hoc within-group comparisons confirmed that both the control boys and the girls with GD showed stronger frontal and parietal



**Table 3** Group differences in brain activation during mental rotation at baseline (session 1)

	ROI	R/L	AAL LABEL	X	Y	Z	N	Zmax	P VALUE
Ctrl Girls > Ctrl Boys	frontal	R	precentral/ inf frontal operculum	52	3	4	50	3,2	<b>.012</b>
			mid-frontal/ frontal superior	26	6	48	174	2,8	.084
	frontal	L	precentral	-24	-9	42	87	3,2	.066
	parietal	R	supramarginal gyrus	55	-28	42	89	3,4	.068
	parietal	L	cuneus	-15	-78	37	164	3,5	<b>.038</b>
precuneus			-12	-67	57	263	3,3	.082	
Ctrl Girls > Girls with GD	frontal	R	precentral / inf frontal operculum	57	6	24	43	3,2	<b>.034</b>

Ctrl = control; GD = Gender Dysphoria; ROI = region of interest; AAL = Anatomic Automated Labeling atlas; x y z = coordinates in Montreal Neurological Institute space; N = number of voxels; Zmax = peak voxel z statistic; R = right hemisphere; L = left hemisphere.

**Table 4** Session effects and session by group interactions

	ROI	R/L	AAL LABEL	X	Y	Z	N	Zmax	P VALUE
<b>Group x session interactions</b>									
Ctrl Boys > Ctrl Girls	frontal	L	supplementary motor area/sup frontal	-15	-3	51	40	3.5	<b>.021</b>
	parietal	L	precuneus/sup occipital	-15	-64	31	77	3.7	<b>.024</b>
			sup parietal/inf parietal	-23	-54	51	121	3.7	<b>.040</b>
Girls with GD > Ctrl Girls	parietal	R	sup parietal/inf parietal	27	-58	61	26	3.5	.057
	parietal	L	cuneus/sup occipital	-15	-79	37	51	4.4	<b>.002</b>
<b>Session (2 &gt;1)</b>									
Ctrl Boys	frontal	R	mid-frontal/precentral	24	-1	48	202	3.5	<b>.016</b>
	frontal	L	sup frontal/precentral	-51	8	34	101	4.0	<b>.004</b>
	parietal	R	sup parietal/angularis	27	-58	48	342	4.0	<b>.013</b>
			supra marginal/ postcentral	58	-27	45	31	4.0	<b>.013</b>
			inf parietal/parietal sup	36	-40	49	106	3.5	.063
	parietal	L	sup parietal/inf parietal	-21	-57	52	1381	4.7	<b>.001</b>
mid-occipital/sup occipital			-26	-73	31	130	4.2	<b>.004</b>	
Girls with GD	frontal	L	precentral /inf frontal triangularis	-56	6	33	61	3.9	<b>.005</b>
			precentral /sup frontal	-30	-6	61	19	3.3	<b>.031</b>
	parietal	R	sup parietal/inf parietal	24	-60	61	300	3.9	<b>.018</b>
	parietal	L	postcentral /sup parietal	-42	-40	57	366	4.5	<b>.001</b>
			sup parietal/precuneus	-17	-67	57	341	3.9	<b>.012</b>

Ctrl = control; GD = Gender Dysphoria; ROI = region of interest; AAL = Anatomic Automated Labeling atlas; x y z = coordinates in Montreal Neurological Institute space; N = number of voxels; Zmax = peak voxel z statistic; R = right hemisphere; L = left hemisphere.



activations at session 2 compared to session 1, whereas no significant effects of session were found in the control girls (see Table 4 and Figure 7.3).

The linear regression analyses in the girls with GD, testing for testosterone treatment-related effects on brain activation that varied according to a) treatment duration and b) cumulative dosage of testosterone, yielded no significant effects within any of the ROIs at our a priori threshold. At a more liberal, whole-brain threshold of  $p < 0.001$  uncorrected, we observed a significant activation cluster in the cerebellum (MNI-coordinates: -24, -42, -38;  $z = 3.5$ ) that was significantly positively correlated with the duration of testosterone treatment. Thus, brain activation in the cerebellum may increase with longer duration of the testosterone treatment. By contrast, the regression analysis for cumulative dosage administered revealed no significant effects.

## Discussion

IN THE PRESENT STUDY, we confirmed that males and females show significant sex differences in brain activation during mental rotation. Control girls had significantly increased right frontal and left parietal brain activation during mental rotation compared to control boys. Similarly, control girls showed increased right frontal brain activation when compared to girls with GD who had not yet started testosterone treatment. Thus, girls with GD showed a priori masculinized brain activation with respect to the frontal brain areas implicated in mental rotation. After 10 months of testosterone exposure, girls with GD showed significantly increased bilateral parietal and left frontal brain activation during mental rotation. We observed a similar pattern of increased frontal and parietal brain activation in the control boys at session 2 (see Figure 3). Our findings thus suggest a testosterone-induced (further) masculinization of brain activation during a visuo-spatial cognitive task in girls with GD.

The fMRI mental rotation task yielded significant activations in a distinct network of brain areas that have repeatedly been reported in studies using mental rotation task paradigms (Zacks 2008). In addition to these robust activations across groups, we found, as expected, significant sex differences in mental rotation associated brain activations. Control girls showed increased brain activation compared to control boys in right inferior frontal areas (pre-central gyrus and frontal inferior operculum), and less pronounced in the left



cuneus (see Table 3). Particularly our finding of significantly stronger inferior frontal activations in girls is in line with previous studies conducted in adult populations (Thomsen et al. 2000; Dietrich et al. 2001; Weiss et al. 2003; Kucian et al. 2005; Hoppe et al. 2012). Sex differences in parietal activations during mental rotation have been less consistently reported. Some studies suggested stronger superior parietal activations in women (Jordan et al. 2002; Weiss et al. 2003; Clements-Stephens et al. 2009), whereas other studies showed significantly stronger inferior parietal (Weiss et al. 2003; Schöning et al. 2007; Hoppe et al. 2012), posterior intra-parietal (Jordan et al. 2002), or superior parietal (Thomsen et al. 2000) activations in men.

The first aim of our study was to investigate whether girls with GD show a more male-typical (similar to experienced gender), rather than female-typical (similar to natal sex) activation pattern during mental rotation. The comparison of control girls to girls with GD at baseline, thus prior to testosterone treatment, revealed a significant effect in the same inferior frontal brain region that was found when control girls were compared to control boys (see Figure 7.2). Thus, girls with GD showed a priori masculinized mental rotation associated brain activations, and thus atypical for their natal sex in terms of visuo-spatial cognitive functioning. Testing girls with GD on GnRHa enabled us to control for possible activational effects of endogenous sex hormones on spatial abilities. In addition, the group comparisons between control boys and girls with GD revealed no significant differences in brain activation during mental rotation, supporting the notion of a priori masculinized cognitive functioning of girls with GD. However, we cannot rule out that the suppression of endogenous gonadal sex steroids may have contributed to the differences found between girls with GD and control girls. In behavioral studies, estrogen treatment in adult men with GD was shown to have detrimental effects on their mental rotation performance (Van Goozen et al. 1995; Slabbekoorn et al. 1999). It may therefore be possible that the girls with GD, in contrast to control girls, were not affected by the inhibiting effects of circulating estrogens on visuo-spatial cognitive functions. Future longitudinal studies should therefore include baseline measurements, thus prior to treatment with GnRHa. Nonetheless, in line with earlier research (Cohen-Kettenis et al. 1998; van Goozen et al. 2002), the present study suggests a masculinization of brain structures associated with visuo-spatial cognitive functions in girls with GD, presumably originating from a critical, pre-/perinatal period of brain sexual differentiation. Although another

< FIGURE 7.2  
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similar neuroimaging study included only adult natal men with GD (Schöning et al. 2010), that study also suggested sex-atypical brain activations during mental rotation in individuals with GD.

The second aim of our study was to investigate whether several months of testosterone treatment in girls with GD would affect their visuo-spatial cognitive functioning. Interestingly, we found significant and very similar group by session interaction effects when comparing control boys with control girls and when comparing girls with GD with control girls. Accordingly, significant within-group effects of session were observed in the control boys and the girls with GD. In the control girls brain activations during mental rotation remained unchanged between sessions. Thus, the brain activation increases in mental rotation-implicated brain areas in the girls with GD mirrored those effects found in the male controls. The control boys, between both test sessions of course grew older (from  $M = 15.9$  to  $M = 17.2$  years of age) and became physically more mature, which is accompanied by an increase in endogenous testosterone secretion (Ankarberg-Lindgren and Norjavaara 2004). In line with our results, Sommer et al. (2008) found overall increased brain activation during mental rotation to be associated with the post-treatment testosterone levels in a group of adult natal men and women with GD. Thus, testosterone indeed seemed to influence visuo-spatial cognition and associated brain activations. In contrast, Carrillo et al. (2010) found no group differences between adult women with GD receiving testosterone treatment and control men or women, which might be related to differences in treatment protocols and the younger age of our still developing participants.

In addition to the activations in our predefined cortical regions of interest, we found that the duration of testosterone treatment correlated positively with activation in the cerebellum, albeit only at a lenient threshold. Other studies have however observed similar cerebellar activations in relation to the mental rotation task (Tagaris et al. 1998; Jordan et al. 2001; Vingerhoets et al. 2001; Schöning et al. 2010; Hoppe et al. 2012), specifically in men (Kucian et al. 2005; Butler et al. 2007), or in relation to serum testosterone levels (Mendrek et al. 2011). Thus, brain regions outside the primary mental rotation network may also be susceptible to the effects of testosterone, thereby affecting visuo-spatial cognitive functioning.

In contrast to previous studies, which showed superior male performance on the mental rotation task (reaction time, accuracy) (Linn and Petersen 1985;



Crucian and Berenbaum 1998; Kimura 2002), we did not find any significant group differences on the behavioral parameters, which might be related to our relatively small group sizes and, thus to a lack of statistical power. Moreover, particularly in the control boys task performance remained stable across sessions, whereas both groups of natal girls showed improvements in accuracy and reaction times at session 2 (see Table 2). Thus, our neuroimaging findings of a testosterone-associated increase in brain activation during mental rotation do not match our behavioral data. We speculate that the underlying cause for the task improvement may be different for the two groups of natal girls. The girls with GD may indeed have benefited from the testosterone treatment in terms of better visuo-spatial performance, as has previously been suggested by Aleman et al. (2004). In the control girls, better performance might be related to motivational motives and the striving to excel at a task which is generally more difficult to accomplish for females. Accordingly, the control girls showed a strong improvement with regard to reaction times, whereas their accuracy scores only improved moderately.

Our results should be viewed in light of some limitations. First, we did not account for any possible effects of menstrual cycle or the use of hormonal contraception, which have previously been shown to affect sex differences in mental rotation performance (Silverman and Phillips 1993; Peters et al. 1995; Dietrich et al. 2001; McCormick and Teillon 2001; Gizewski et al. 2006; Schöning et al. 2007). However, these effects of fluctuating endogenous hormone levels on visuo-spatial performance were relatively small. In addition, the control girls were tested randomly according to the phase of their menstrual cycle and about half of them were using hormonal contraceptives. Therefore, we do not expect that any systematic differences in circulating sex hormone levels might have affected our results.

In addition, it should be noted that sexual orientation might present a confounding factor. Maylor et al. (2007) and Peters et al. (2007) showed that performance on the mental rotation task varied as a function of sexual orientation: homosexual men performed worse compared to heterosexual men, whereas lesbian women excelled in mental rotation performance compared to heterosexual women. The majority of natal females with GD are gynephilic (Lawrence 2010; Nieder et al. 2011; Cerwenka et al. 2014), which was also found in our group of adolescent gender dysphoric girls. Although the effects of sexual orientation have only been shown for behavioral responses and have not been investigated



using neuroimaging studies of visuo-spatial cognitive functions, we cannot rule out that a similar cerebral activation pattern during mental rotation in control boys and girls with GD might be associated with their shared sexual preference, rather than their shared gender identity. However, several previous studies observed these effects of sexual orientation on mental rotation performance primarily in men, and found very moderate or even negligible effects in women (Sanders and Ross-Field 1986; Wegesin 1998; Rahman and Wilson 2003; Rahman et al. 2004), while others failed to find any relationship between sexual orientation and visuo-spatial performances at all (Tuttle and Pillard 1991; Gladue and Bailey 1995; Hall and Kimura 1995). Thus, the existing literature suggests that any influence of sexual orientation on mental rotation performance mainly applies to (adult) men and we therefore believe that such effects in our young natal female population are likely to be small.

Finally, an alternative explanation for our findings that girls with GD showed similar visuo-spatial cognitive functions as control boys may be that both groups share similar interests and preferences for certain hobbies and activities, such as video-games, sports, etc. Thus, the differences found between control girls and girls with GD may also be related to their differential experiences with visuo-spatial tasks and may therefore reflect, at least in part, training effects.

In conclusion, the current study provides new insights into the differential organizational and activational effects of testosterone on visuo-spatial cognitive functioning. We found sex-atypical mental rotation-associated brain activations in adolescent girls with GD, suggesting a masculinization of brain structures associated with visuo-spatial cognitive functions. Moreover, the treatment-effect results indicate that testosterone may induce significant changes in the neural correlates of visuo-spatial cognitive functions.

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