Research Paper

Male-typical visuospatial functioning in gynephilic girls with gender dysphoria — organizational and activational effects of testosterone

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Background: Sex differences in performance and regional brain activity during mental rotation have been reported repeatedly and reflect organizational and activational effects of sex hormones. We investigated whether adolescent girls with gender dysphoria (GD), before and after 10 months of testosterone treatment, showed male-typical brain activity during a mental rotation task (MRT). **Methods:** Girls with GD underwent fMRI while performing the MRT twice: when receiving medication to suppress their endogenous sex hormones before onset of testosterone treatment, and 10 months later during testosterone treatment. Two age-matched control groups participated twice as well. **Results:** We included 21 girls with GD, 20 male controls and 21 female controls in our study. In the absence of any group differences in performance, control girls showed significantly increased activation in frontal brain areas compared with control boys (p_{FWE} = 0.012). Girls with GD before testosterone treatment differed significantly in frontal brain activation from the control girls (p_{FWE} = 0.034), suggesting a masculinization of brain structures associated with visuospatial cognitive functions. After 10 months of testosterone treatment, girls with GD, similar to the control boys, showed increases in brain activation in areas implicated in mental rotation. **Limitations:** Since all girls with GD identified as gynephilic, their resemblance in spatial cognition with the control boys, who were also gynephilic, may have been related to their shared sexual orientation rather than their shared gender identity. We did not account for menstrual cycle phase or contraceptive use in our analyses. **Conclusion:** Our findings suggest atypical sexual differentiation of the brain in natal girls with GD and provide new evidence for organizational and activational effects of testosterone on visuospatial cognitive functioning.

Introduction

The mental rotation task (MRT),[1,2] a visuospatial working memory task, has consistently been shown to elicit robust sex differences in performance, with men outperforming women.[3,4] Accordingly, fMRI studies have found that men and women use different cerebral networks when they have to determine whether 2 differently rotated 3-dimensional figures are identical or mirrored. Stronger superior parietal activations during mental rotation have been observed in men, whereas women recruit (inferior) frontal and temporal brain areas more than men.[5]

The classical theory of organizational and activational effects of sex hormones on the brain[6] assumes that functional (i.e., behavioural) differences between men and women reflect sex differences at the structural (morphological) level, which have been established ("organized") during prenatal development. Sex differences in visuospatial cognition evolve during early development under the organizational influence of sex hormones.[7] Evidence for these early hormonal effects on later visuospatial abilities comes from studies in individuals with congenital adrenal hyperplasia,[8–10] idiopathic hypogonadotropic hypogonadism,[11] or complete androgen insensitivity syndrome.[12] These conditions are all characterized by aberrant androgen action from early development onwards. In addition, sex differences in mental rotation performance have already been found in children,[13–16] suggesting that sex differences in spatial functioning observed in adulthood reflect sex differences in exposure to androgens during the perinatal period of sexual differentiation.

However, some studies have failed to observe sex differences in mental rotation functions in children in contrast to older participants,[17–19] which suggests that postnatal factors, such as puberty, cognitive development and experience, may also affect the sex-specific development in mental rotation functioning. Significant effects of age and age × sex interactions in studies

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Submitted Apr. 27, 2015; Revised Nov. 19, 2015; Revised Dec. 20, 2015; Accepted Dec. 20, 2015; Early-released Apr. 12, 2016

DOI: 10.1503/jpn.150147

on mental rotation performance during adolescence[20,21] have suggested that activational effects of sex hormones, starting at puberty, reinforce sex differences in visuospatial functioning. Furthermore, several studies[22,23] have indicated effects of gonadal hormone fluctuations in girls on visuospatial cognitive functioning.

Individuals with gender dysphoria (GD; DSM-5[24]) are characterized by distress due to a profound feeling of incongruence between their natal sex and experienced gender. It has been hypothesized that atypical levels of pre- and perinatal sex steroids during a critical period of sexual differentiation of the brain may be involved in the development of GD.[25]

Neuropsychological studies involving adults with GD have yielded some support for both organizational and activational effects of testosterone on mental rotation performance. Treatment-naive study participants with GD performed comparably to their experienced gender control groups (e.g., performance in women with GD was similar to control men),[26,27] and cross-sex hormone (CSH) treatment (i.e., natal women receive testosterone, natal men receive estrogen) improved performance in natal women and had detrimental effects in natal men.[28–30] However, other studies failed to observe early or late sex hormone–dependent changes or differences in spatial abilities between individuals with GD and controls.[31–33]

Three fMRI studies[34–36] investigated sex-typical (in accordance with natal sex) and sex-atypical (in accordance with experienced gender) brain functioning during mental rotation in treatment-naive individuals with GD as well as in participants receiving CSH treatment. However, these reports focused mostly on adult men with GD and thus on the activational effects of estrogen treatment, whereas the association between testosterone and neuroimaging correlates of spatial cognition in women with GD remains understudied.

In the present prospective fMRI study, the first aim was to investigate whether a carefully selected, highly homogeneous (in terms of GD onset age, sexual orientation, dosage and type of the CSH treatment) group of adolescent natal girls with GD would show a male- or female-typical brain activation pattern during an fMRI MRT before the start of the testosterone treatment. At the Center of Expertise on Gender Dysphoria at the VU University Medical Center in Amsterdam, the Netherlands, 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 receive CSH treatment.[37,38]

Our second aim was to investigate the effects of testosterone on MRT performance and associated brain functioning. Thus, girls with GD participating in the present study were tested twice: shortly before receiving testosterone while their endogenous sex hormones were suppressed, and then again 10 months later while receiving testosterone treatment. We hypothesized that girls with GD, based on the assumption that they have undergone a more masculinized early neuronal sexual differentiation, would show male-typical mental rotation functioning (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 when receiving testosterone (activational effects).

Methods

Participants

Adolescent girls with GD who had been gender dysphoric since childhood were recruited via the Center of Expertise on Gender Dysphoria. Age-matched controls were recruited via several secondary schools in the Netherlands and by inviting friends of the participants with GD. Exclusion criteria for participation in the study were any form of neurologic or psychiatric disorder and continuous psychotropic medication use. When scanned for the first time (session 1), girls with GD had been treated monthly with 3.75 mg of triptorelin (Decapeptyl-CR, Ferring) by injection for on average 24 (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 (range 6–15) months. All girls with GD either received a testosterone ester mixture (Sustanon 250 mg/mL Merck Sharp & Dohme bv) every 2 weeks or testosterone undecanoate (Nebido, 250 mg/mL, Bayer) every 12 weeks. The starting dosage varied with the patient's age. Until the age of 16.5 years, the starting dosage was 25 mg/m^2 body surface area every 2 weeks. When older than 16.5 years the dosage was 75 mg every 2 weeks.[39] Controls were exposed to their endogenous sex hormones during both test sessions. Female controls were tested randomly according to their menstrual cycles, and we assessed use of hormonal contraceptives, but this was not an exclusion criterion.

Procedure

Before the session 1 fMRI scan, all participants underwent a neuropsychological assessment and olfactory function test (results published elsewhere[40]) lasting approximately 90 min. Participants completed 4 subtests (arithmetic, vocabulary, picture arrangement and block design) of the Wechsler Intelligence Scale for Children[41] or, if older than 16 years, the Wechsler Intelligence Scale for Adults.[42] Each 4-subtest sum score was converted to an individual's estimated IQ. We assessed sexual orientation by asking whether the participant had ever been in love with somebody and whether that person was a boy or a girl. Pubertal stages were assessed in the control participants by means of self-report,[43] and in girls with GD by a pediatric endocrinologist (D.T.K.) as part of their clinical assessments.[44,45]

Participants were instructed on the fMRI paradigm and performed 2 practice trials of the MRT before the scan. The fMRI session also included 2 other fMRI tasks,[40] a resting state and diffusion tensor imaging scan. The whole scanning session lasted approximately 1 hour.

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.

Hormone assessments

At both test sessions for the control groups and at session 2 for the girls with GD, testosterone levels were measured in saliva, which provides an index of the free (i.e., unbound, or biologically available) fraction of testosterone in circulation.[46] Participants were asked to collect saliva samples at home by salivating at least 1 mL into a polypropylene tube, directly after waking up on the day of the MRI scan. Samples were brought to the clinic and stored at –80°C until analysis. Testosterone levels were determined with an isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) method. For further details on the analysis see the study by Bui and colleagues.[47]

Functional MRI mental rotation

Participants were presented with Shepard and Metzler–type 3-dimensional (3D) white drawings on a black background taken from the mental rotation stimulus library, provided by Peters and Battista.[48] In the mental rotation condition, participants were presented 40 pairs of 3D shapes, with 1 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 9 different angles, with at least 80° difference between the 2 presented shapes. Participants had to indicate (by pressing a button) whether the 2 shapes were identical or mirror images. During the control condition 1 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. Stimuli were presented using a classical block design, with 16 alternating rotation/control blocks, and each block contained 5 mental rotation or control trials. Presentation duration of each stimulus varied depending on the participant's performance, with a maximum stimulus presentation of 20 s. Outcome parameters were the percentage of trials correctly identified and mean reaction time per trial.

Imaging protocol

All scans for session 1 were performed on a 3.0 T GE Signa HDxt scanner. A gradient-echo echo-planar imaging sequence was used for functional imaging. The parameters included a 24 cm^2 field of view (FOV), repetition time (TR) of 2100 ms, echo time (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 coregistration with the functional images we obtained a T_1-weighted scan (3D FSPGR sequence, 25 cm^2 FOV, 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. Thus, for all session 2 scans, approximately 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.

Data analysis

Behavioural data

Demographic, self-report, and performance data as well as the hormone assessments were analyzed using the Statistical Package for the Social Sciences, version 20 (SPSS Inc.). Differences in group characteristics and performance were analyzed using 1-way analysis of variance (ANOVA). A repeated-measures ANOVA was conducted to assess session effects in MRT performance, with accuracy and mean reaction time per trial as within-subjects factors and group as a between-subjects factor, including IQ as a covariate. The significance level was set at $p < 0.05$.

Neuroimaging data

We performed fMRI data analysis using SPM8 software (Wellcome Department of Imaging Neuroscience, Institute of Neurology at the University College London) implemented in Matlab R2012b (MathWorks Inc.). Functional images were slice-timed, realigned to the mean image, and coregistered with the individual anatomic image. Applying the 'New Segment' and 'Create Template' options of the DARTEL toolbox, structural images were segmented. Then we used grey matter and white matter images to create 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 using an 8 mm full-width at half-maximum 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 mm displacement).[49] Together with the 6 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.

We conducted second-level random effects analyses, entering all individual contrast images (mental rotation > control condition) from session 1 into a 1-way ANOVA in order to test for sex differences (control boys > / < control girls), and to determine whether girls with GD at baseline (i.e., during hormonal suppression and before CSH treatment) compared with the control boys and control girls, showed a female- or male-typical mental rotation activation pattern.

By means of a flexible factorial design, testing within-group differences between sessions and group × session interaction effects, we investigated the effects of the testosterone treatment in girls with GD (session 2 v. 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. In case of significant interactions, we used post hoc paired-sample t tests to explore within-group session effects.

In order to further explore the effects of the testosterone treatment on visuospatial brain functioning in girls with GD, we extracted brain activation (using MarsBaR[50]). We specifically focused on those clusters in which girls with GD showed male-typical effects and investigated correlations

between MRT brain activation and testosterone levels of the girls with GD for these clusters.

According to a meta-analysis of neuroimaging studies on brain regions implicated in mental rotation,[51] we focused our imaging analyses on predefined regions of interest (ROI), encompassing the intraparietal sulcus, the precentral sulcus and the inferior frontal sulcus. These 3 bilateral ROIs were selected from Nielsen and Hansen's[52] volume of interest BrainMap database. Using the MarsBaR tool,[50] the anatomic ROIs were masked with the control groups' MRT main effect (applying a whole-brain threshold of $p < 0.05$, family-wise error [FWE]–corrected) in order to create 4 separate ROIs: the precentral and the inferior frontal sulcus combined were defined as "frontal ROI," for the right (4240 mm^3) and left hemisphere (5728 mm^3), respectively; the right parietal (20744 mm^3) and left parietal (16288 mm^3) ROI. All group comparisons were covaried 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

Participant characteristics

Twenty-one adolescent girls (mean age 16.1 ± 0.8 yr) with GD, 20 control boys (mean age 15.9 ± 0.6 yr) and 21 control girls (mean age 16.3 ± 1.0 yr) participated in the study. Among the girls with GD, 14 received Sustanon every 2 weeks and 7 received Nebido every 12 weeks during the testosterone treatment phase. One control girl and 4 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.

The demographic, self-report data and testosterone levels of participants are presented in Table 1. The IQ scores of the girls with GD were significantly lower than those of both control groups, therefore we included IQ scores as a covariate in all further between-groups analyses. At session 1, all participants were in pubertal stage 4 or higher (1 = prepubertal, 5 = postpubertal). For the subscale pubic hair growth, the control girls on average rated themselves half a stage lower than the control boys and the girls with GD, which resulted in a significant effect for the overall group comparison ($F_{2,59} = 3.9$, $p = 0.027$). With regard to genital and breast development, there were no group differences, therefore we decided not to include puberty stage as a covariate in the further analyses. Saliva samples of 2 control girls and 1 control boy were missing, and 1 control girl had an extremely high testosterone value in comparison to all other control girls and was therefore excluded from all analyses. There were no differences in mean testosterone levels between session 1 and session 2 for the control groups. The post-treatment testosterone levels of the girls with GD were comparable to those of the control boys. At session 1, 11 of 21 control girls, and at session 2, 15 of 20 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).

Behavioural data

One-way ANOVA yielded no significant group differences in MRT performance (Table 2). The repeated-measures ANOVA, corrected for group differences in IQ, revealed a significant main effect of session in mental rotation accuracy ($F_{1,53} = 11.9$, $p = 0.001$). No main effect of group or any group × session interaction was observed. Cohen d effect sizes suggested moderate to strong improvements in reaction time

Table 1: Demographic and clinical characteristics of study participants

Characateristic	Session	Group; mean ± SD*			Statistic	p value
		Girls with GD	Control girls	Control boys		
No. of participants	1	21	21	20	—	—
	2	21	20	16	—	—
Age, yr	1	16.1 ± 0.8	16.3 ± 1.0	15.9 ± 0.6	$F_{2,59} = 1.1$	0.34
	2	17.1 ± 0.7	17.6 ± 0.8	17.2 ± 0.7	$F_{2,54} = 1.9$	0.16
Puberty stages†						
Pubic hair growth	1	4.7 ± 0.6	4.2 ± 0.7	4.7 ± 0.7	$F_{2,59} = 3.9$	0.027
Genital development‡/ breast development§	1	4.1 ± 1.1	4.1 ± 0.8	4.1 ± 0.8	$F_{2,59} < 0.1$	0.98
IQ	1	100.5 ± 12.7	110.3 ± 14.7	113.4 ± 14.5	$F_{2,59} = 5.1$	0.009
Sexual orientation	1	100% gynephilic	100% androphilic	100% gynephilic	—	—
Testosterone levels, median (range), pmol/L	1	—	39.0 (13–130)¶	307.0 (158–552)	—	—
	2	285.0 (130–545)	30.0 (13–109)¶	323.5 (186–630)**	—	—

GD = gender dysphoria; SD = standard deviation.
*Unless indicated otherwise.
†Pubertal stages were assessed using the 5-point (1 = prepubertal, 5 = post-pubertal) Tanner Maturation Scale.
‡Applies to natal boys.
§Applies to natal girls.
¶$n = 19$.
**$n = 15$.

and accuracy for both the girls with GD and the control girls (mainly in reaction times), whereas the performance of the control boys remained stable (Table 2).

Neuroimaging data

During mental rotation all 3 groups showed widespread task-related bilateral activations, recruiting parieto-occipital and frontal networks (Fig. 1).

Group differences in mental rotation

The between-group comparisons at baseline (session 1) revealed significant sex differences in mental rotation–associated brain activation. Control girls showed several clusters of increased activation compared with control boys in the right frontal and left parietal ROIs. The reverse contrast, testing for any increased activation during mental rotation in control boys versus control girls yielded no significant

Table 2: Performance data for the mental rotation task

		Group; mean ± SD*				
Variable	Session	Girls with GD	Control girls	Control boys	Statistic	*p* value
% correct	1	66.7 ± 15.9	67.0 ± 11.6	70.2 ± 10.7	$F_{2,59} = 0.5$	0.64
	2	74.2 ± 9.0	71.7 ± 8.2	71.6 ± 10.3	$F_{2,54} = 0.5$	0.60
Cohen *d*†		–0.59	–0.48	–0.14		
RT/trial	1	8.0 ± 2.2	8.2 ± 1.5	8.1 ± 1.6	$F_{2,59} = 0.04$	0.96
	2	6.7 ± 2.1	6.8 ± 1.7	7.5 ± 2.0	$F_{2,54} = 1.0$	0.39
Cohen *d*†		0.62	0.90	0.35		

GD = gender dysphoria; RT = reaction time; SD = standard deviation.
*Unless indicated otherwise.
†Effect sizes were calculated for group means at session 1 versus session 2 using the pooled SD of the 2 means.

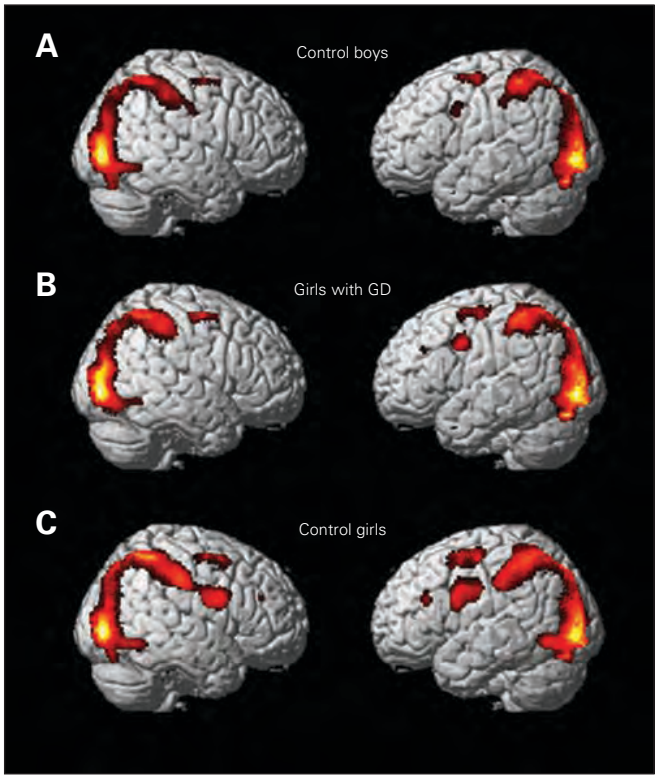

Fig. 1: Brain activation pattern during mental rotation at session 1 in (**A**) control boys, (**B**) girls with gender dysphoria (GD) and (**C**) control girls. Statistical parametric maps were rendered on an SPM8 template image showing the left and right hemisphere in sagittal view. For illustrative purposes, whole brain results are displayed at an uncorrected threshold of $p < 0.005$.

effects. Comparing control girls and girls with GD revealed a significant activation in the right frontal ROI, similar to the sex difference observed between the control groups (Fig. 2 and Table 3). No other between-group differences were significant.

Testosterone-induced effects

Two of the contrasts testing group × session interactions (control boys > control girls and girls with GD > control girls) revealed significant effects in the left frontal and both parietal ROIs (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 activations at session 2 than at session 1, whereas no significant changes in brain activation between sessions were found in the control girls (Table 4 and Fig. 3).

None of the regression analyses revealed any significant correlations between male-typical MRT brain activation and post-treatment saliva testosterone levels in the girls with GD.

Discussion

In the present study, we demonstrated sex differences in brain activation during mental rotation. Control girls had significantly increased right inferior frontal (precentral gyrus and frontal inferior operculum) and left parietal (cuneus) activation during mental rotation compared with control boys. Similarly, control girls showed increased right frontal activation compared with girls with GD who had not yet started testosterone treatment. Thus, girls with GD showed a priori masculinized mental rotation–associated brain activations, and were thus atypical for their natal sex in terms of visuospatial cognitive functioning. 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 masculinized cognitive functioning of girls with GD. Testing girls with GD on GnRHa enabled us to control for possible activational effects of endogenous sex hormones on spatial abilities in this group. 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 behavioural studies, estrogen treatment in adult men with GD was shown to have detrimental effects on their mental rotation performance.[25,26] It is therefore possible that the girls with GD, in contrast to control girls, were not affected by the inhibiting effects of circulating estrogens on visuospatial cognitive functions. Nonetheless, in line with previous research,[23,24] our study suggests a masculinization of

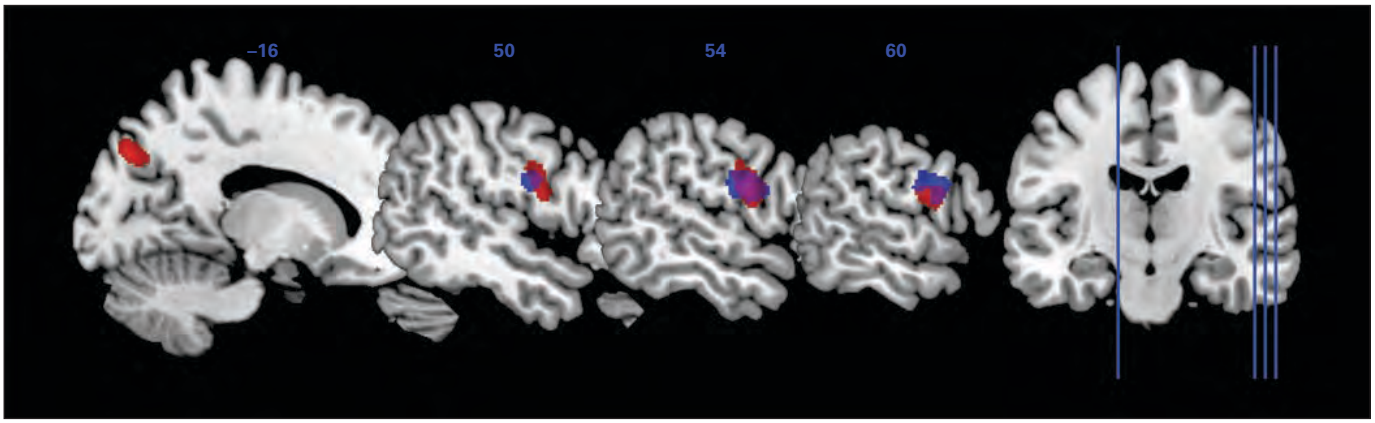

Fig. 2: Between-group differences in brain activation in right frontal and left parietal areas during mental rotation at session 1. Red = control girls > control boys; blue = control girls > girls with gender dysphoria. Numbers indicate x axis coordinates in Montreal Neurological Institute space, displayed in sagittal view. See Table 3 for further details.

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

Comparison	ROI		AAL label	MNI space *x*, *y*, *z*	No. of voxels	Z_{max}	p_{FWE} value
Control girls > control boys	Frontal	R	Precentral/inferior frontal operculum	52, 3, 24	50	3.2	0.012
			Mid-frontal/frontal superior	26, 6, 48	174	2.8	0.08
	Frontal	L	Precentral	−24, −9, 42	87	3.2	0.07
	Parietal	R	Supramarginal gyrus	55, −28, 42	89	3.4	0.07
	Parietal	L	Cuneus	−15, −78, 37	164	3.5	0.038
			Precuneus	−12, −67, 57	263	3.3	0.08
Control girls > girls with GD	Frontal	R	Precentral/inferior frontal operculum	57, 6, 24	43	3.2	0.034

AAL = automated anatomic labelling; FWE = family-wise error; GD = gender dysphoria; L = left hemisphere; MNI = Montreal Neurological Institute; R = right hemisphere; ROI = region of interest.

brain structures associated with visuospatial cognitive functions in girls with GD, presumably originating from a critical perinatal period of sexual differentiation in the brain.

Our prospective design of testing girls with GD before and after 10 months of testosterone treatment and also testing male and female controls twice, allowed for the specific investigation of the effects of testosterone on MRT-associated brain activation. After 10 months of testosterone exposure, girls with GD showed significantly increased bilateral parietal and left frontal activation during mental rotation. We observed a similar pattern of increased frontal and parietal activation in the control boys at session 2, whereas in the control girls, brain activations during mental rotation remained unchanged between sessions. Interestingly, we found significant and very similar group × session interaction effects when comparing control boys with control girls and when comparing girls with GD with control girls. Thus, the increase in parietal and frontal activation in session 2 compared with session 1 in the girls with GD mirrored those effects found in the male controls. The control boys, of course, aged (from a mean age of 15.9 to 17.2 yr) and matured physically between both test sessions, which is accompanied by an

Table 4: Session effects and session × group interactions

Effect	ROI		AAL atlas	MNI space *x*, *y*, *z*	No. of voxels	*Z*max	p_{FWE} value
Group × session interactions							
Control boys > control girls	Frontal	L	Supplementary motor area/superior frontal	–15, –3, 51	40	3.5	0.021
	Parietal	L	Precuneus/superior occipital	–15, –64, 31	77	3.7	0.024
			Superior parietal/inferior parietal	–23, –54, 51	121	3.7	0.049
Girls with GD > control girls	Parietal	R	Superior parietal/inferior parietal	27, –58, 61	26	3.5	0.06
	Parietal	L	Cuneus/superior occipital	–15, –79, 37	51	4.4	0.002
Session (2 > 1)							
Control boys	Frontal	R	Mid-frontal/precentral	24, –1, 48	202	3.5	0.016
	Frontal	L	Superior frontal/precentral	–51, 8, 34	101	4.0	0.004
	Parietal	R	Superior parietal/angularis	27, –58, 48	342	4.0	0.013
			Supra marginal/postcentral	58, –27, 45	31	4.0	0.013
			Inferior parietal/superior parietal	36, –40, 49	106	3.5	0.06
	Parietal	L	Superior parietal/inferior parietal	–21, –57, 52	1381	4.7	0.001
			Mid-occipital/superior occipital	–26, –73, 31	130	4.2	0.004
Girls with GD	Frontal	L	Precentral/inferior frontal triangularis	–56, 6, 33	61	3.9	0.005
	Parietal	R	Superior parietal/inferior parietal	24, –60, 61	300	3.9	0.018
	Parietal	L	Postcentral/superior parietal	–42, –40, 57	366	4.5	0.001
			Superior parietal/precuneus	–17, –67, 57	341	3.9	0.012

AAL = automated anatomic labelling; FWE = family-wise error; GD = gender dysphoria; L = left hemisphere; MNI = Montreal Neurological Institute; R = right hemisphere; ROI = region of interest.

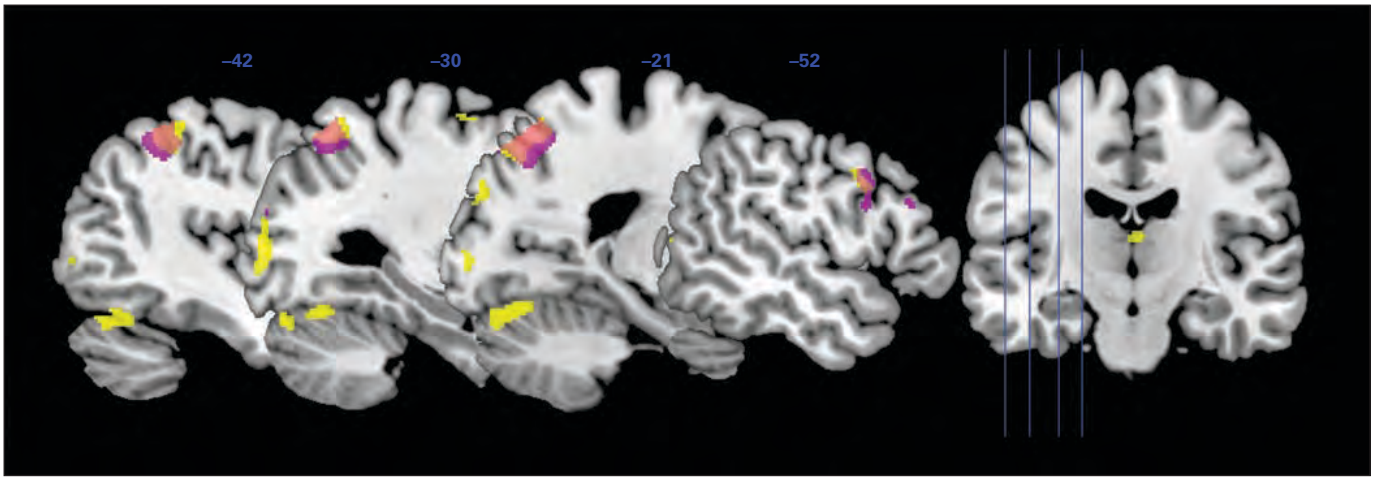

Fig. 3: Clusters of significant increases in brain activation during mental rotation for session 2 compared with session 1. Left parietal and left frontal regions are shown. Yellow = session 2 > session 1 in girls with gender dysphoria; purple = session 2 > session 1 in control boys. See Table 4 for further details.

increase in endogenous testosterone secretion.[53] The increase in parietal and frontal brain activations in the male controls may therefore be testosterone-dependent as well.

Similar to our results, Sommer and colleagues[36] found natal women with GD to have increased brain activation in MRT-implicated brain areas after 3 months of testosterone treatment. However, this finding did not reach statistical significance, possibly owing to the limited sample size of only 6 women with GD. In contrast, Carrillo and colleagues[34] found no group differences between 19 adult women with GD receiving testosterone treatment and control men or women. In their study, pretreatment data were not reported, therefore within-group effects of the testosterone treatment could not be determined. The authors noted that they did not control for menstrual cycle effects, which might have influenced their results.

At odds with our findings, another study testing the effects of testosterone treatment in 9 postmenopausal women indicated decreased parietal activation during mental rotation after 26 weeks of transdermal testosterone treatment.[54]

The present study, suggesting a masculinization of the functional neuroanatomy of visuospatial working memory in natal girls with GD, are in line with 2 recent reports showing testosterone treatment effects on morphological brain measures: cortical thickness, subcortical volumes,[55] and white matter microstructure.[56]

Next to the group comparisons, we aimed to explore the association between the testosterone treatment and visuospatial brain functioning in girls with GD by means of regression analyses. However, we found no significant correlations between their post-treatment testosterone levels and their male-typical parietal and frontal activation during mental rotation at session 2, which suggests that within-group variations in brain activation in girls with GD were not related to the activational effects of current testosterone levels.

In contrast to previous studies that showed superior male performance (reaction time, accuracy) on the MRT,[3,57] we did not find any significant group differences on the behavioural parameters. However, sex differences in brain activation need not necessarily be reflected in sex differences on a behavioural level, as has been shown by Jordan and colleagues.[58] Moreover, particularly in the control boys MRT task performance remained stable across sessions, whereas both groups of natal girls showed improvements in accuracy and reaction times at session 2 (Table 2). Thus, our neuroimaging findings of a testosterone-associated increase in parietal and frontal activation during mental rotation do not match our MRT performance data. We speculate that the underlying cause for the task improvement may be different for the 2 groups of natal girls. The girls with GD may indeed have benefited from the testosterone treatment in terms of better visuospatial performance, as has been suggested previously by Aleman and colleagues.[59] In the control girls, better performance might be related to motivation and 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 improved only moderately.

Limitations

Our results should be viewed in light of some limitations. First, by design, the groups differed with regard to the puberty suppressing treatment at baseline. Therefore, we cannot rule out that any differences in brain activation or behaviour between the girls with GD and the control girls may have been due to the hormonal suppression.

Second, we did not account for possible effects of menstrual cycle or the use of hormonal contraception, which have previously been shown to affect sex differences in mental rotation performance.[60–62] However, these effects of fluctuating endogenous hormone levels on visuospatial performance were relatively small. In addition, the control girls were tested randomly according to the phase of their menstrual cycles, 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.

Third, it should be noted that sexual orientation might present a confounding factor. Peters and colleagues[63] and Maylor and colleagues[64] showed that performance on the MRT varied as a function of sexual orientation: homosexual men performed worse than heterosexual men, whereas lesbian women excelled in mental rotation performance compared with heterosexual women. The majority of natal women with GD are gynephilic,[65–67] which was also found in our group of adolescent girls with GD. However, effects of sexual orientation have been shown only for behavioural responses and have not been investigated using neuroimaging studies of visuospatial cognitive abilities. Moreover, the effects of sexual orientation on mental rotation performance were observed primarily in men, whereas only moderate or even negligible effects were found in women.[68–70] 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 visuospatial cognitive functions as control boys may be that both groups share similar interests and preferences for certain hobbies and activities, such as video games and sports. Thus, the differences found between control girls and girls with GD may also be related to their differential experiences with visuospatial tasks and may therefore reflect, at least in part, training effects.

Conclusion

We found sex-atypical mental rotation–associated brain activations in adolescent girls with GD, suggesting a masculinization of brain structures associated with visuospatial cognitive functions. Moreover, our prospective fMRI study provides new insights into the differential organizational and activational effects of testosterone on visuospatial cognitive functioning.

Acknowledgments: The authors thank Ms. Willeke Menks for her help with participant recruitment and support during the fMRI and neuropsychological data acquisition and Mr. Ton Schweigmann for his efforts in coordinating and supporting the fMRI data acquisition.

This work was supported by a VICI grant (453-08-003) from the Dutch Science Foundation (Nederlandse Organisatie voor Wetenschappelijk Onderzoek) to J. Bakker. J. Bakker is a senior research associate of the Belgian Fonds National de la Recherche Scientifique.

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Competing interests: None declared.

Contributors: S. Burke, P. Cohen-Kettenis and J. Bakker designed the study. S. Burke and D. Klink acquired the data, which S. Burke, B. Kreukels, P. Cohen-Kettenis, D. Veltman and J. Bakker analyzed. S. Burke and J. Bakker wrote the article, which all authors reviewed and approved for publication.

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