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Cortical activation during mental rotation in male-to-female and female-to-male transsexuals under hormonal treatment

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fMRI

Summary There is strong evidence of sex differences in mental rotation tasks. Transsexualism is an extreme gender identity disorder in which individuals seek cross-gender treatment to change their sex. The aim of our study was to investigate if male-to-female (MF) and female-to-male (FM) transsexuals receiving cross-sex hormonal treatment have different patterns of cortical activation during a three-dimensional (3D) mental rotation task. An fMRI study was performed using a 3-T scan in a sample of 18 MF and 19 FM under chronic cross-sex hormonal treatment. Twenty-three males and 19 females served as controls. The general pattern of cerebral activation seen while visualizing the rotated and non-rotated figures was similar for all four groups showing strong occipito-parieto-frontal brain activation. However, compared to control males, the activation of MF transsexuals during the task was lower in the superior parietal lobe. Compared to control females, MF transsexuals showed higher activation in orbital and right dorsolateral prefrontal regions and lower activation in the left prefrontal gyrus. FM transsexuals did not differ from either the MF transsexual or control groups. Regression analyses between cerebral activation and the number of months of hormonal treatment showed a significant negative correlation in parietal, occipital and temporal regions in the MF transsexuals. No significant correlations with time were seen in the FM transsexuals. In conclusion, although we did not find a specific pattern of cerebral activation in the FM transsexuals, we have identified a specific pattern of cerebral activation during a mental 3D rotation task in MF transsexuals under cross-sex hormonal treatment that differed from control males in the parietal region and from control females in the orbital prefrontal region. The hypoactivation in MF transsexuals in the parietal region could be due to the

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hormonal treatment or could reflect a priori cerebral differences between MF transsexual and control subjects.

1. Introduction

Transsexualism [World Health Organization, 1993 (ICD-10)], also known as gender identity disorder (GID) in adulthood or adolescence [American Psychiatric Association, 2000 (DSM-IV-TR)], is characterized by a strong and persistent cross-gender identification, accompanied by persistent discomfort with the biological sex or a sense of inappropriateness in the gender role of that sex, and is usually accompanied by the desire to make the body as congruent as possible with the desired sex through cross-sex hormone treatment and surgery (for review see Cohen-Kettenis and Gooren, 1999; Green, 2002; Gooren, 2006).

There is strong evidence of sex differences in spatial abilities (Voyer et al., 1995). It has been found that three-dimensional (3D) mental rotation tests produce the most consistent sex differences in favor of males (Vandenberg and Kuse, 1978; Linn and Petersen, 1985; Voyer et al., 1995; Karádi et al., 2003; Peters, 2005). Mental rotation tasks require deciding whether two shapes with different angulations are identical. Response times increase as the angle of disparity between the two shapes increases. The explanation for this finding is that an image of the shape has to be mentally rotated and then superimposed over the reference shape in order for the subject to decide whether the shapes are identical or not (Kosslyn and Brown, 1995). The neural substrates of mental rotation were investigated by Cohen et al. (1996) using fMRI. They described the cerebral activation in ten subjects performing the Shepard and Metzler (1971) mental rotation task with 3D shapes, and found consistent foci of activation in bilateral parietal regions and in middle frontal gyrus; they concluded that the neural structures most involved in mental rotation were the extrastriate visual regions of the superior parietal lobes, the V5/MT region engaged in motion, and the frontal eye fields, probably involved in the scanning of complex visual images. Curiously, they did not find right hemisphere predominance.

It has been suggested that sex differences in spatial abilities are related to the level of prenatal androgens (Kimura, 1999). Studies on spatial abilities in congenital adrenal hyperplasia (CAH) seem to support this idea (Resnick et al., 1986; Berenbaum, 2001). However, a more recent work (Hines et al., 2003; Malouf et al., 2006) found that CAH females were not different from unaffected females. Moreover, testosterone and estradiol are important in the modulation of spatial abilities throughout life. Intermittent androgen suppression adversely affects mental rotation (Cherrier et al., 2003). Higher levels of salivary testosterone are associated with lower error rates and faster responses in mental rotation tests (Hooven et al., 2004). In addition, Aleman et al. (2004) demonstrated that a single administration of testosterone improves 3D mental rotation abilities in young women. In contrast, estradiol has been reported to have a negative effect on spatial abilities in men (Kozaki and Yasukouchi, 2008) and women during the menstrual cycle (Phillips and Silverman, 1997; Hausmann et al., 2000). However, other studies found no evidence of changes in spatial abilities during the menstrual cycle (Epting and Overman, 1998) or in MF transsexuals under estrogen treatment (Miles et al., 2006).

Functional magnetic resonance imaging (fMRI) has been used to investigate the cerebral basis of sexual differences in spatial tasks. Gur et al. (2000), using a 2D rotation task, showed that males have a left activation that is not seen in females. Studying subjects with a similar performance in the mental rotation task, Jordan et al. (2002) observed a significantly increased activation in the left motor cortex in males compared to females and increased activation of parietal and temporal regions in females compared to males. Weiss et al. (2003), in a sample of university students selected for high performance accuracy in the mental rotation test, found that males activated the inferior parietal lobe more than females; on the other hand, females activated the superior parietal lobes and the right inferior frontal gyrus more than males. Hugdahl et al. (2006) reported that males have increased right parietal activation while the females, in addition, show inferior frontal activation. They suggested that males may be biased towards a coordinate processing approach, and females biased towards a serial, categorical processing approach.

Table 1 Characteristics of the samples and group comparisons.

	Male-to-female transsexuals (n = 18)	Female-to-male transsexuals (n = 19)	Control females (n = 19)	Control males (n = 23)	F/χ^2	p values
Age	37.28 ± 5.560	33.74 ± 8.272	32.58 ± 8.585	31.48 ± 6.466	F = 2.29	0.085
Education*					χ^2 = 4.18	0.24
Mental rotation test						
Accuracy	22.79 ± 3.79	21.28 ± 5.31	21.58 ± 3.45[a]	23.91 ± 4.21	F = 4.04	<0.05
Reaction time**	3044 ± 653	3306 ± 539	2904 ± 587[b]	3198 ± 618	F = 4.20	<0.05

[a] Significant differences with respect to control male group; $p < 0.05$.
[b] Significant differences with respect to FM transsexual group; $p < 0.05$.
* Educational level is classified on a scale running from 1 (illiterate) to 7 (university graduate).
** Reaction time was measured in milliseconds.

Table 2 Levels of hormones and sex reassignment treatments in transsexuals.

	Male-to-female transsexuals	Female-to-male transsexuals
Months of hormonal treatments	115.61 ± 80.76	60.00 ± 42.66
17β-Estradiol (IU/L)	37.14 ± 28.16	28.43 ± 22.34
Testosterone (ng/dL)	145.56 ± 261.26	646.56 ± 536.72
T index	11.35 ± 17.36	65.50 ± 50.01
SSB globulin (nmol/L)	79.46 ± 56.67	38.26 ± 13.75
Types of surgical treatments	6[a]	18[b] 9[c]

Hormonal results are expressed as mean ± standard deviation. Normal estradiol levels: males (10–41 pg/mL); females in follicular phase (22–55 pg/mL) and luteal phase (68–196 pg/mL). Normal testosterone levels: adult males (275–850 ng/dL) and females (10–80 ng/dL). Normal free testosterone index: adult males (38–123%) and females (1–7%). Normal levels of sex steroid binging globulin: adult males (10–60 nmol/L) and females (35–135 nmol/L).

[a] Vaginoplastia.

[b] Mastectomy.

[c] At least one of the following surgical treatments (histerectomy, faloplastia, metaidoioplastia).

The effects of hormones on spatial abilities have also been investigated in transsexual patients because of possible changes in mental rotation due to cross-sex hormonal treatments. With respect to 3D mental rotation, Slabbekoorn et al. (1999) found that untreated MF transsexuals had better performance than untreated FM transsexuals but after ten months of treatment the differences were reversed. However, they did not replicate their previous results using a 2D rotation test (Van Goozen et al., 1995). A study with untreated GID patients found that the pattern of mental rotation was consistent with that of their biological sex and not with that of their gender identity (Haraldsen et al., 2003). Later, the same group communicated that cross-sex hormone treatment did not change the sex-sensitive mental rotation ability since results in the cross-sex hormone-treated transsexual patients were identical to those in the controls (Haraldsen et al., 2005). Moreover, there is another study signalling that estrogen, associated with cross-sex change treatment in MF transsexuals, has no influence on mental rotation (Miles et al., 2006).

There are only two fMRI studies of mental rotation in transsexuals. One did not obtain significant effects, probably because of a small sample size (Sommer et al., 2008). In a more recent study Schöning et al. (2009) studied a sample of treated and untreated MF subjects and found that the two transsexual groups had increased activation in the temporo-occipital regions and decreased activation in the left parietal lobe compared to control men. This investigation did not include FM transsexuals in the design.

Taking into account all the above literature, the present work was designed to compare the patterns of cortical activation during a 3D mental rotation task in a large sample of MF and FM transsexuals under chronic cross-sex hormonal treatments, with heterosexual males and females serving as controls.

2. Methods

2.1. Subjects

The study sample was comprised of 18 MF and 19 FM transsexuals, and heterosexual control groups of 23 males and 19 females (Table 1). All participants were right-handed.

The transsexual groups were recruited from the Gender Identity Unit (GIU) at the Hospital Clinic of Barcelona (Spain) (see Gómez-Gil et al., 2009a). Diagnostic assessment of transsexualism followed the revised fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR; American Psychiatric Association, 2000) and the tenth revision of the International Classification of Diseases (ICD-10; World Health Organization, 1993) and was made after several semi-structured interviews with two mental health professionals (psychiatrist and psychologist) (Gómez-Gil et al., 2009b).

Since transsexualism is a heterogeneous category (Blanchard et al., 1987; Blanchard, 1989; Lawrence, 2005), all transsexuals in our study were selected because they presented early-onset gender nonconformity (before puberty), were erotically attracted to the same biological sex, and were interested in sex reassignment or had had surgery. This group corresponds to the one typically referred to as "homosexual type" according to Blanchard et al. (1987) [see also Blanchard, 1989; Smith et al., 2005; but see Gooren, 2006]. Sexual orientation was established by asking what partner (a man, a woman, both or neither) the patient would prefer or feel attraction to if they were completely free to choose and the body did not interfere.

According to the GIU hormonal protocol, MF transsexuals received treatment with estrogens (oral ethinyl estradiol or transdermal 17β-estradiol) associated, if vaginoplasty had not been performed, to the anti-androgen cyproterone acetate. FM transsexuals received either intramuscular depot injections of testosterone esters every 2–4 weeks or daily transdermal testosterone as patches or gel. The hormone administration route and schedule was heterogeneous, frequently defined by patients' personal preferences. The hormonal levels of transsexuals were controlled by routine hospital testing every six months and hormonal data from the routine analysis closest in time to the fMRI were assessed for the purpose of this study; Table 2 shows hormonal data and surgical treatments. Competitive chemoluminiscent immunoassays were run for estradiol (ADVIA Centaur, Siemens; sensitivity: 10 pg/mL) and testosterone (Cobas, Roche; sensitivity 10 ng/dL); a sandwich type chemoluminiscent immunoassay was run for sex steroid binding hemoglobulin (SHBG) (Cobas, Roche; sensitivity: 0.4 nm/L). The free

Table 3 Contrasts between groups in cortical activation in a three-dimensional mental rotation task.

Locations	Talairach coordinates				
	x	*y*	*z*	*t*	Cluster size
Control males > control females					
Precuneus (left) BA 31	**0**	**−37**	**10**	**4.94**	**62**
Gyrus lingualis (left) BA 30	−6	−44	0	4.01	
Control males > male-to-female transsexuals					
Superior parietal lobule (left) BA 7	**−24**	**−74**	**42**	**5.19**	**166**
Superior parietal lobule (left) BA 7	−21	−61	53	4.09	
Precuneus (left) BA 7	−9	−53	52	4.06	
Inferior parietal lobule (right) BA 7	**30**	**−35**	**49**	**4.71**	**64**
Superior parietal lobule (right) BA 7	36	−38	60	4.22	
Superior parietal lobule (right) BA 7	**21**	**−49**	**58**	**4.26**	**45**
Control females > male-to-female transsexuals					
Postcentral gyrus (left) BA 2	**−41**	**−27**	**37**	**4.35**	**36**
Male-to-female transsexuals > control females					
Orbital gyrus (right) BA 11	**6**	**58**	**−5**	**4.64**	**77**
Gyrus frontalis superior part medialis (left) BA 10	−15	55	3	3.99	
Gyrus frontalis superior part (left) BA 9	−12	56	11	3.38	
Caudate nucleus (right) BA 24	**21**	**27**	**11**	**4.27**	**38**
Caudate nucleus (right) BA 24	15	24	18	4.06	
Caudate nucleus (right) BA 33	21	18	18	3.95	
Orbital gyrus (right) BA 11	**18**	**49**	**−2**	**4.18**	**33**

Location *x*, *y* and *z* coordinates are based on the atlas of Talairach and Tournoux (1988). Uncorrected statistical threshold at $p < 0.001$.

testosterone index was calculated as a percentage, dividing testosterone (nmol/L) by SHBG (nmol/L).

The control volunteers were recruited from the community by advertisement and were screened by a psychiatrist to rule out any current or recent endocrine disorder or psychiatric history, the latter by means of the Spanish Version 5.0.0 (Bobes et al., 1997) of the International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). Controls were comparable by age and educational level (Table 1). Only heterosexual controls were included in the study.

After a full explanation of the study, all subjects gave written informed consent to a protocol conducted in accordance to the Declaration of Helsinki and approved by the ethics committee at the Hospital Clinic of Barcelona.

2.2. MRI acquisition

All MR scanning was performed on a Magneton Trio Tim 3.0 T SIEMENS (Erlangen, Germany). Functional data were collected using an echo-planar (EPI) sequence sensitive to blood oxygen level dependent (BOLD) contrast [TR repetition time (TR)/echo time (TE) = 2000/29 ms; field of view (FOV) = 24 × 24 cm, 128 × 128 pixel matrix; flip angle = 90°; slice thickness 3 mm and 20 axial slices per scan]. One run consisting of 280 volumes was acquired during the experiment.

Following fMRI scan, and in order to aid in the localization of functional data, a high resolution T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) 3D MRI sequence in sagittal plane with the following parameters was acquired: TR/TE = 2300/2.98 ms; TI 900; FOV = 25 × 25; 256 × 256 pixel matrix; 1 mm slice thickness.

2.3. fMRI mental rotation task

The experimental paradigm consisted of a block design composed of activation and baseline mental rotation tasks presented alternatively. The stimuli were chosen from figures in the Vandenberg-Kuse MRT Check List (Vandenberg and Kuse, 1978) and we selected the stimuli that were most discriminatory between genders from the Karádi et al. (2003) study; there were 80 items. We used the Presentation 10.1 program version (Neurobehavioral System, USA) to develop the stimuli task and VisuaStim Digital MRI Compatible High Resolution Stereo 3D glasses (Resonance Technology Inc.) to present stimuli inside the scanner. Participants were first trained outside the scanner to mentally rotate figures into alignment: for that purpose 8 stimuli were presented. In the fMRI study 10 blocks of rotated figures and 10 blocks of non-rotated figures were presented. Each stimulus lasted 5000 ms; the interstimuli interval was 1000 ms, all items were presented in 20 blocks of 4 items each. In addition, each block was preceded by a 4000 ms instruction and every block lasted 28 s. Each block presented 2 alternate conditions: 2 equal stimuli and 2 different stimuli. Image acquisition for the entire experimental sequence took 9 min and 20 s. Participants were required to indicate whether or not the figures were the same, albeit rotated, by pressing a button with their right hand. In the baseline situation, subjects were asked to do the same but were advised that the figures were not rotated.

2.4. fMRI preprocessing

After reconstructing the images, volume image data were saved in Analyze format (http://www.analyzedirect.com).

Data were then pre-processed and analyzed using statistical parametric mapping software (SPM5, Wellcome Department of Cognitive Neurology; Queens Square, London, UK). The images of each subject were corrected for motion and realigned to remove any minor motion-related signal change. The ranges of subjects' motion parameters were checked for a movement of less than 0.5 mm in spatial translation or 2° rotations in any angular direction. The images generated from these data were spatially normalized to the SPM5 EPI template image in the MNI atlas space. During spatial normalization all scans were re-sampled to 3 mm^3 isotropic voxels. Additionally, low-frequency noise was removed with a high-pass filter (128 s) applied to the fMRI time series at each voxel. Finally, the images were spatially smoothed with an 8 mm full width half maximum (FWHM) isotropic Gaussian kernel.

2.5. Demographic and statistical analyses of the mental rotation task

Data were analyzed using the SPSS V.16 statistical software package for Windows. In order to confirm that patients and controls were comparable we analyzed age using ANOVA and educational characteristics using a chi-squared analysis. The level of significance was set at $p < 0.05$.

For the mental rotation task, we recorded the number of figure stimuli correctly identified (accuracy) and response times calculating the mean reaction time (in milliseconds) for the correct trials. Mean correct scores and reaction times were compared between groups using one-way analyses of variance (ANOVAs). In order to test the differences between the four groups with respect to accuracy and reaction time during the task, a 2 (sex: male, female) × 2 (condition: control, transsexuals) analysis of variance was conducted. Tukey's post hoc comparisons were used to study the differences between the groups.

2.6. fMRI data analysis

We estimated the condition-specific effects (activation > baseline) on a single subject level using the General Linear Model approach. After completing the individual-level analysis, an SPM hierarchical full factorial two-stage analysis method was used to examine group-level activation comparing the 4 groups: control males, control females, MF transsexuals and FM transsexuals. Correlation analyses between brain activation and months of treatment were performed separately for FM and MF transsexuals. The threshold at voxel level value was set at uncorrected $p < 0.001$. The cluster extent threshold was considered at greater than 30 voxels. The location of activations in the Montreal Neurological Institute (MNI) coordinates was converted to Talairach space (Talairach and Tournoux, 1988).

3. Results

3.1. Mental rotation task

3.1.1. Task performances

With respect to the number of correct responses, statistically significant sex by condition interaction indicated the existence of sex differences. The results are displayed in Table 1, control males performed better than control females. No differences were observed between control females and the FM or MF transsexual groups. Although not quite significant, control males performed slightly better than MF transsexuals, but they did not differ from FM transsexuals. No other significant interactions were seen.

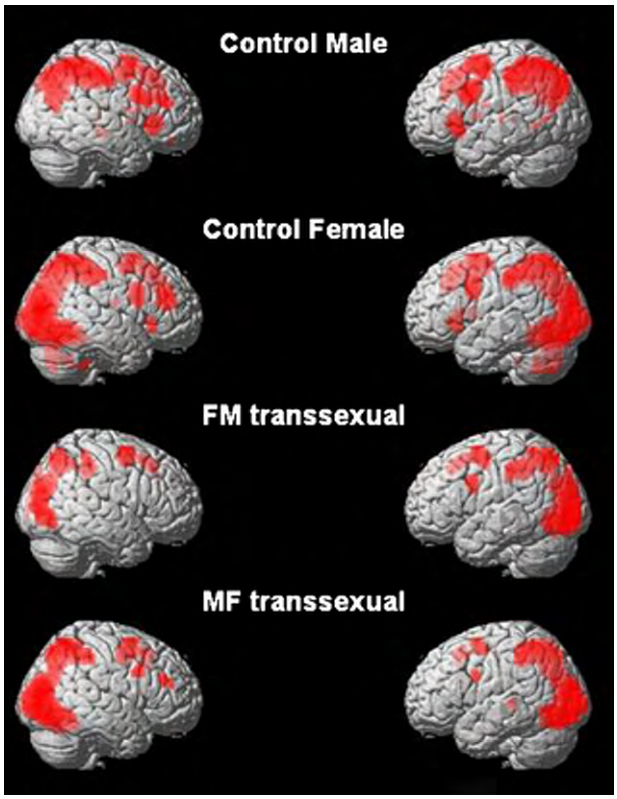

Figure 1 Statistical parametric maps of brain activation in males, females, male-to-female and female-to-male transsexuals during a mental rotation task. Sagittal views of the patterns of cerebral activation in the contrast between rotated and non-rotated figures in the four groups of subjects analyzed separately. The frontal, parietal and occipital region activation is similar for the four groups. The uncorrected statistical threshold is $p < 0.001$.

3.1.2. Reaction time

In relation to response latency, the ANOVA analysis produced a statistically significant sex by condition interaction (see Table 1). Post hoc analysis indicated that control females were quicker to respond than FM transsexuals. No differences were seen between the remaining groups.

3.2. fMRI results

The pattern of cerebral activation seen in the contrast between rotated and non-rotated figures was similar in all four groups. We observed a strong pattern of bilateral activation in the parietal, occipital and frontal lobes (Fig. 1).

The contrast between groups in the mental rotation task showed significant differences between the MF transsexual group and the two control groups. As can be seen in Fig. 2 and

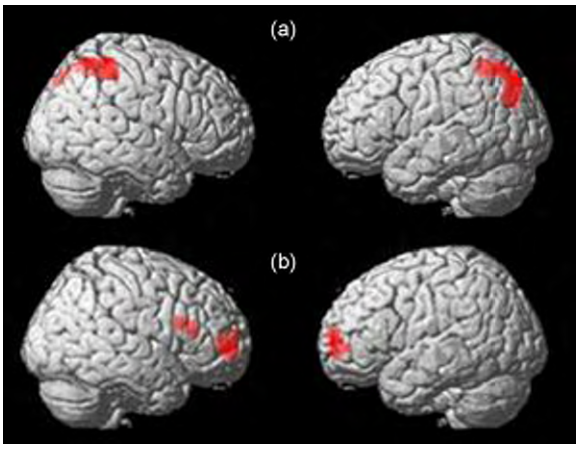

Figure 2 Statistical parametric maps of brain activation in the contrast between (a) control males and male-to-female transsexuals and (b) control females and male-to-female transsexuals during a mental rotation task. (a) Decreased superior parietal cortical activation can be observed in the male-to-female transsexuals compared to males. (b) Activation is increased in the male-to-female transsexuals compared to control females in the prefrontal cortex. The red color indicates the regions that reached statistical significance. The uncorrected statistical threshold for the two panels is $p < 0.001$.

Table 3, MF transsexuals' activation during the task was lower in the superior parietal lobe than in control males. In contrast, MF transsexuals showed increased activation in the orbital and right dorsolateral prefrontal regions compared to control females (Fig. 2, Table 3). In the left postcentral gyrus MF transsexuals showed less activation than control females. Male and female controls differed in the activation of the left hemisphere precuneus, gyrus lingualis and superior temporal gyrus (Table 3). FM transsexuals did not show any significant difference in the contrasts between groups.

The simple regression analyses performed between cerebral activation and the number of months of hormonal treatment in MF and FM transsexuals showed a significant negative correlation in the MF transsexuals. As can be seen in Fig. 3, in these subjects, the correlations in the right hemisphere are seen in the superior parietal lobe and the posterior part of the temporal lobe. In the left hemisphere, the significant correlations are in the superior parietal lobe. No significant correlations were seen in the FM group.

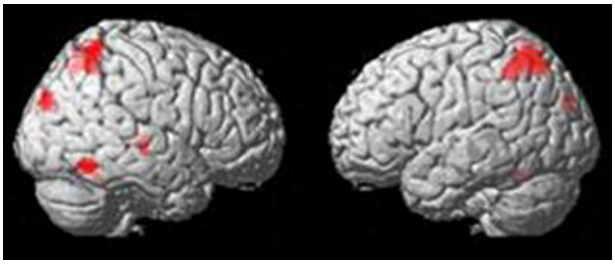

Figure 3 Statistical parametric maps showing the cerebral areas that achieved significant negative correlation between cerebral activation and months of cross-sex hormonal treatments in male-to-female transsexuals. As can be observed, the largest clusters involve the superior parietal regions. This location coincides with the hypoactivation seen in the male-to-female transsexuals compared to male controls (see Fig. 2).

4. Discussion

The aim of our study was to investigate the pattern of cortical activation in chronic hormonally treated MF and FM transsexuals. The main finding of our work was that MF transsexuals differed from both male and female controls. In contrast, FM transsexuals did not differ from the control groups and, in addition, there was no difference between either transsexual group.

Our findings in the mental rotation task agree with the previous literature showing better performance in males compared to females, (Thomsen et al., 2000; Jordan et al., 2002; Seurinck et al., 2004; Hugdahl et al., 2006) and coincide with the literature showing that spatial abilities are one of the functions that best reflect sex differences in cognition (Fisher and Pellegrino, 1988; Peters et al., 1995; Phillips and Silverman, 1997; Astur et al., 2004; Parsons et al., 2004). However, other authors (Jordan et al., 2002; Weiss et al., 2003; Hugdahl et al., 2006) have not found sex differences in the subjects' performance during the fMRI paradigm of mental rotation. It should be taken into account that Weiss et al. (2003) selected the subjects according to their high performance accuracy in the mental rotation test, so, obviously, no difference in the fMRI task accuracy could be expected in their sample. Although we found significant sex differences in the mental rotation task, they were not as large as those reported by others (Halari et al., 2006). The smaller size of the sex differences in our study might be due to the stimulus presentation design used and the fact that subjects were first trained outside the scanner to mentally rotate figures.

We did not find significant differences between control females and transsexuals, but MF transsexuals tended to be somewhat less accurate than control males. The decrease in performance in this group may be due to their low levels of testosterone resulting from their feminizing cross-sex hormone treatment. In this sense, there are a substantial number of papers suggesting that testosterone could be responsible for the more accurate male than female performance in spatial abilities such as mental rotation (for review Janowsky, 2006; Zitzmann, 2006). Moreover, it has been reported that a single administration of testosterone improves visuospatial ability in women (Aleman et al., 2004). In reaction time, we only observed that FM transsexuals were slower than our control females. There is a report in the literature showing that testosterone levels are associated with faster responses in men (Hooven et al., 2004) but in our case this did not occur.

The pattern of brain activation seen in each separate group (contrast between rotated and non-rotated figures) is similar to that reported in the literature (Cohen et al., 1996; Vingerhoets et al., 2002; Halari et al., 2006; Hugdahl et al., 2006). All the groups showed an intense cerebral activation involving the posterior occipital and parietal regions and, similarly to other authors (Halari et al., 2006; Schöning et al., 2007, 2009), we also observed frontal activation.

The contrast between groups showed that MF transsexuals differed from both of the control groups regarding the brain region recruited to solve the task. The MF group showed less activation bilaterally than control males in the parieto-occipital regions. According to the model of Kosslyn (see Kosslyn and Brown, 1995), the superior parietal region is one of the components of the network for mental rotation (Cohen et al., 1996). The role of the superior parietal region in spatial perception has been demonstrated in experimental lesion studies performed in non-human primates (Ungerleider and Haxby, 1994). Similar evidences have come from focal lesions produced by strokes and tumors in humans (Vallar, 2007).

MF transsexuals differed from control females bilaterally in the prefrontal orbital regions, with the transsexual showing greater activation than their female controls. The differences between MF transsexuals and control females were located in a more anterior region. The orbitofrontal cortex is crucial for emotion and decision-making tasks (Bechara, 2004). Jordan et al. (2002) found that females exhibited significant bilateral activation in the intraparietal sulcus and the superior and inferior parietal lobe, as well as in the inferior temporal gyrus, while males showed significant activation in the left intraparietal sulcus, the left superior parietal lobe and the right parieto-occipital sulcus. These authors explained the differences in brain activation as the expression of differences in cognitive strategies. In this line, it was reported that males showed significantly stronger parietal activation than females while the latter showed greater activation in the right frontal lobe than males (Weiss et al., 2003). Hugdahl et al. (2006) also reported that males predominantly presented parietal activation while females, in addition, showed frontal basal activation.

Although we found that MF transsexuals differ from male and female controls, FM transsexuals do not show a specific pattern of cerebral activation since they did not differ from the MF and control groups. This result is surprising and very difficult to explain. It could be speculated that the fact that transsexual groups are receiving opposite cross-sex treatments might place FM transsexuals in an intermediate position in cerebral activation that could mask possibly significant differences. Another possibility is that this ''middle'' position could be the result of the interaction between structural brain differences and the hormonal treatment; that might be the case if the hormonal treatment was acting on the brain structures implicated in mental rotation in an already feminized or masculinized brain.

In our study we only found two clusters of decreased activation in left visual associative regions in female controls compared to male controls. Halari et al. (2006) found that males outperformed females in the mental rotation task, but the cortical activation was comparable in the two groups during mental rotation.

To the best of our knowledge, there are only two previous studies on the cerebral patterns of activation during mental rotation in transsexuals. Sommer et al. (2008) did not find group differences between transsexuals. However, Schöning et al. (2009) compared untreated MF transsexuals, treated MF transsexuals and control males and found that the two MF transsexual groups exhibited significantly less activation of the left parietal cortex and increased activation in the temporo-occipital regions. The decreased activation that we observed in MF transsexuals in the parietal regions is coincident with the last study, but the effect was bilateral in our subjects.

It is well known that sex hormones act on the mammalian brain. Association cortices, which are involved in complex cognitive functions, including spatial abilities in human and non-human primates, have androgen (Puy et al., 1995; Abdelgadir et al., 1999; Finley and Kritzer, 1999; Beyenburg et al., 2000; Bezdickova et al., 2007) and estrogen (Österlund et al., 2000a,b) receptors. Curiously, although the transsexual groups are receiving the opposite hormone treatment they do not differ in their pattern of cerebral activation. The role of estradiol in mental rotation has been discussed in the literature. Dietrich et al. (2001) found that women during the mid-luteal phase (high estradiol levels) showed increased parietal activation. Moreover, Gizewski et al. (2006) reported women show greater activation in the right temporal and frontal cortices. However, Epting and Overman (1998) observed that there was no evidence that performances differed with the menstrual cycle. Miles et al. (2006) suggested that estrogen treatment associated with sex reassignment treatment in MF transsexuals has little or no influence on mental rotation. In addition, in a revision of the recent literature, Hines (2006) concluded that prenatal levels of testosterone do not appear to influence visuospatial abilities that normally show sex differences. However, we found a significant negative correlation between cerebral activation in posterior cerebral regions and the length of the treatment in MF but not FM transsexuals. The regions that showed this correlation partially coincide with the regions in which MF transsexuals showed less activation than control males. This suggests that the differences would result from the hormonal treatment. Our design does not allow us to determine whether the decrease in testosterone levels and/or the increases in estradiol levels are the origin of the correlation. There are recent data in the literature that support a role by testosterone treatment in the performance of mental rotation. Sommer et al. (2008) reported that the cerebral activation during mental rotation did not increase during treatment, but post-treatment testosterone levels (three months later) correlated to total activation during mental rotation in FM transsexuals. In our study, we did not find any correlation in the FM group after many months of testosterone treatment. In male subjects, Schöning et al. (2007) observed a significant correlation between activation levels in the left parietal lobe and testosterone levels. Moreover, females' brain activation in frontal and parietal areas was significantly correlated with estradiol.

The hypoactivation in MF transsexuals in the parietal region could be due to the hormonal treatment or might reflect a priori differences between MF transsexual and control subjects. Although hormones seem to be related to the differential pattern observed in MF transsexuals, these differences could also emerge from differences in the cerebral structure. Recently, in a morphometric study, Luders et al. (2009) studied untreated MF transsexuals, male and female controls and reported that MF transsexuals only differed from male controls in the right putamen; the authors suggest that regional gray matter in MF transsexuals is more similar to the pattern found in men than in women. Schöning et al. (2009), using an fMRI design to test differences in spatial cognition in untreated and treated MF transsexuals and control men, found differences in the activation of the

left parietal cortex that remain stable, suggesting that cognitive changes are not a relevant side-effect of hormonal treatment. However, this study, like our study, was not longitudinal.

The present research has some limitations. First, since all transsexuals were receiving hormonal treatment, this study cannot establish whether the differences are a direct effect of the hormonal treatment or whether they emerge from underlying differences in the cerebral structure. Comparative studies between transsexuals before and after treatment are needed. Second, we did not control for menstrual cycle effects. Increased cortical activation in the mid-luteal phase has been reported in fMRI studies on mental rotation (Dietrich et al., 2001; Gizewski et al., 2006). Finally our quantitative analyses do not allow us to detect possible sexual differences in the use of strategies to solve the mental rotation task.

In conclusion, we have identified a specific pattern of cerebral activation during mental rotation in MF transsexuals under cross-sex hormonal treatment that differs from control males in the parietal region and from control females in the orbital prefrontal region. Longitudinal designs are needed to resolve the question of whether the hypoactivation in MF transsexuals in the parietal region and the absence of a specific cerebral activation pattern in FM transsexuals could be due to the cross-sex hormonal treatment or might reflect a priori differences.

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Conflicts of interest

The authors do not have financial or personal conflicts of interest in this area.

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