Journal of Psychiatric Research 45 (2011) 199–204

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

White matter microstructure in female to male transsexuals before cross-sex hormonal treatment. A diffusion tensor imaging study

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ARTICLE INFO

Article history:
Received 4 March 2010
Received in revised form
10 May 2010
Accepted 10 May 2010

Keywords:
Diffusion tensor imaging
Transsexualism
Sex differences
Superior longitudinal fasciculus
Forceps minor
Corticospinal tract

ABSTRACT

Background: Some gray and white matter regions of the brain are sexually dimorphic. The best MRI technique for identifying subtle differences in white matter is diffusion tensor imaging (DTI). The purpose of this paper is to investigate whether white matter patterns in female to male (FtM) transsexuals before commencing cross-sex hormone treatment are more similar to that of their biological sex or to that of their gender identity.

Method: DTI was performed in 18 FtM transsexuals and 24 male and 19 female heterosexual controls scanned with a 3 T Trio Tim Magneton. Fractional anisotropy (FA) was performed on white matter fibers of the whole brain, which was spatially analyzed using Tract-Based Spatial Statistics.

Results: In controls, males have significantly higher FA values than females in the medial and posterior parts of the right superior longitudinal fasciculus (SLF), the forceps minor, and the corticospinal tract. Compared to control females, FtM showed higher FA values in posterior part of the right SLF, the forceps minor and corticospinal tract. Compared to control males, FtM showed only lower FA values in the corticospinal tract.

Conclusions: Our results show that the white matter microstructure pattern in untreated FtM transsexuals is closer to the pattern of subjects who share their gender identity (males) than those who share their biological sex (females). Our results provide evidence for an inherent difference in the brain structure of FtM transsexuals.

1. Introduction

Gender identity disorders (GID) are characterized by persistent cross-gender identification and discomfort with the individual's assigned gender (American Psychiatric Association, 2000). The disorders are manifested by cross-dressing and a search for hormonal and surgical sex reassignment (SR) to the desired anatomical sex. Transsexualism is an extreme form of GID. The etiology of GID is unknown but biological variables may contribute to gender identity variations (Cohen-Kettenis and Gooren, 1999; Swaab, 2004).

Structural brain differences in transsexuals have been addressed by postmortem anatomical as well as by in vivo neuroimaging studies. With respect to the former, it was shown in subcortical structures that the central nucleus of the bed nucleus of the stria terminalis (BSTc), which is important for the male sexual behavior (Claro et al., 1995), is female in size (Zhou et al., 1995) and in neuron number (Kruijver et al., 2000) in male-to-female (MtF) transsexual subjects. These structural differences between MtF transsexuals and their male and female controls were not influenced by changes in sex hormone levels in adulthood and were not related to sexual orientation (Kruijver et al., 2000; Zhou et al., 1995). Additionally, one of the interstitial nuclei of the anterior hypothalamus (INAH3) is larger in men than in women. The volume and number of neurons in the INAH3 of MtF transsexuals was similar to that of control females and this feminization was not due to estrogens treatment (Garcia-Falgueras and Swaab, 2008). The latter study

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doi:10.1016/j.jpsychires.2010.05.006

included one FtM transsexual subject; the INAH3 volume and number of neurons was within the male control range (Garcia-Falgueras and Swaab, 2008).

Although brain MRI structural and functional studies may help to understand transsexualism, few studies have yet been made. The only available structural study (Luders et al., 2009) analyzed MRI data of not yet cross-sex hormone treated MtF transsexuals in order to verify whether their gray matter volumes resembled those of subjects who shared either their biological sex (males) or their gender identity (females). They reported that regional gray matter variation in untreated MtF transsexuals is more similar to the pattern found in males than in females. However, MtF transsexuals show a significantly larger volume of regional gray matter in the right putamen than do control men (Luders et al., 2009).

MRI functional studies examinated the brain while performing tasks, such as mental rotation, in which males and females consistently differ (Kimura, 1999). There are only three fMRI studies of mental rotation in transsexuals. One longitudinal study reported that activation during mental rotation did not increase during cross-sex hormone treatments (Sommer et al., 2008), probably because of the small sample size. However, in a sample of treated and untreated MtF it was found that both transsexual groups had increased activation in the temporo-occipital regions and decreased activation in the left parietal lobe, compared to control men. The authors suggested that are *a priori* differences between males and MtF transsexuals (Schöning et al., 2009). Also in a mental rotation task, comparing chronically treated FtM and MtF transsexuals to male and female controls, we found a parietal hypoactivation in the MtF transsexuals, but no significant differences for FtM transsexuals (Carrillo et al., 2010).

Cerebral activation due to visual erotic stimuli in MtF transsexuals before any treatment has been investigated by means of fMRI (Gizewski et al., 2009). These authors found an activation patter in MtF transsexuals that is similar to the one of females. They concluded that MtF transsexuals show a tendency of female-like cerebral processing in transsexualism.

A couple of MRI studies have focused on white matter in transsexuals. No differences for the whole corpus callosum (CC) or splenium region between transsexuals and controls were reported (Emory et al., 1991). However, using a sophisticated MRI measure of CC shape, it was concluded that the pattern of CC shape in transsexuals is closer to that in individuals whit the same gender identity than to that in individual with the same biological sex (Yokota et al., 2005).

Diffusion Tensor Imaging (DTI) is the most suitable technique to detect subtle changes in the white matter of patients with schizophrenia, depression, obsessive-compulsive disorders, autism or developmental dyslexia (Nucifora et al., 2007). DTI has been used to investigate sex differences in adults (Westerhausen et al., 2003; Huster et al., 2009) and in developmental studies (Schmithorst et al., 2008).

Almost all of the postmorten anatomical (Garcia-Falgueras and Swaab, 2008; Kruijver et al., 2000; Zhou et al., 1995) and MRI (Luders et al., 2009) studies have been focused on the gray matter of MtF transsexuals and little attention has been paid to white or gray matter in FtM transsexuals. Thus, the purpose of the present study was to test if the pattern of the white matter microstructure in FtM transsexuals was congruent with their biological sex or with their gender identity before they underwent cross-sex hormonal treatment. We measured FA as an indicator of white matter coherence and axonal organization. To the best of our knowledge there are no previous studies in the literature describing white matter microstructure in FtM transsexuals.

2. Materials and methods

2.1. Subjects

Subjects were 18 untreated FtM transsexuals from the Gender Identity Unit (GIU) at the Hospital Clinic of Barcelona, with 24 male and 19 female controls recruited by advertisement (see Table 1). All participants were right handed.

The prevalence rate of transsexualism in Catalonia is 1:21,031 males and 1:48,096 females and the sex ratio is 2.6 (Gómez-Gil et al., 2005, 2009a). Diagnostic assessment of transsexualism followed the revised fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2000) and the tenth revision of the International Classification of Diseases (World Health Organization, 1993) and was made after several semi-structured socio-demographic, clinical and psychiatric interviews with two mental health professionals (psychiatrist and psychologist) (Gómez-Gil et al., 2009b).

All FtM transsexuals selected had early-onset gender nonconformity (before puberty), were erotically attracted to females, and wanted sex reassignment (Gómez-Gil et al., 2009b). This group corresponds to the one typically referred to as “homosexual type” (Blanchard et al., 1987; Smith et al., 2005; but see Gooren, 2006). Sexual orientation in patients 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.

All patients were not hormonally treated, and meet the eligibility and readiness criteria for hormone therapy according the Standards of Care of the Harry Benjamin Gender Dysphoria Association (HBGDA; Meyer et al., 2002). Additionally, all patients have initiated cross-sex hormonal therapy with androgens after the MRI scanning. The hormonal levels of the untreated FtM transsexual group were obtained before the study (Table 1).

The healthy control volunteers were recruited from the community by advertisement and were evaluated by a psychiatrist, using the Spanish Version 5.0.0 (Bobes et al., 1997) of the International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998), to

Table 1
Characteristics of the sample and group comparisons.

	FtM transsexuals[a] ($n = 18$)	Control Females ($n = 19$)	Control Males ($n = 24$)	F	p values
Age (years)	28.24 ± 10.61	31.22 ± 6.09	33.00 ± 8.22	1.50	0.23
Hormonal Levels[b]					
Testosterone (ng/dl)	47.97 ± 21.81				
Free testosterone index (%)	3.63 ± 3.00				
Sex steroid binding globulin (nmol/L)	54.71 ± 28.08				
17-β-estradiol (pg/ml)	115.70 ± 86.93				

[a] Female to male transsexuals (FtM). Results are expressed as mean ± standard deviation.
[b] 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 binding globulin: adult males (10–60 nmol/L) and females (35–135 nmol/L). Normal estradiol levels: males (10–41 pg/mL); females: follicular phase (22–55 pg/mL), luteal phase (68–196 pg/mL).

select controls without any psychiatric history. Only heterosexual controls were included in the study. The male controls have a gender identity as a man and a sexual orientation toward women (similar to our FtM patients). Control women have a gender identity as a woman and a sexual orientation toward men (the opposite to the both previous groups). We consider that both control groups are appropriate. Controls and FtM were comparable by age (Table 1).

Study exclusion criteria were having taken any hormone, psychotropic medication and/or illegal drug.

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

2.2. Image acquisition

MRI scanning was performed with a SIEMENS Trio Tim Magneton (Erlangen, Germany) at 3 T. All patients underwent axial DTI (Singleshot diffusion weighted EPI, $b = 1000$ mm^2/s, 64 directions, TE = 94 ms, TR = 9300 ms, flip angle 90, slice thickness = 2 mm, providing 1.97 mm in-plane resolution). In addition, to aid in locating diffusion tensor data, a high-resolution T1-weighted magnetization-prepared rapid gradient-echo (MP-RAGE) 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 resulting in a 1 mm isotropic acquisition voxel.

2.3. DTI analysis

2.3.1. Tract-based spatial statistics (TBSS)

Individual FA processing of diffusion tensor data was performed using the FSL version 4.1.2 (Smith et al., 2004, 2006). Following eddy current correction using the FMRIB Diffusion Toolbox (FDT), non-brain voxels were extracted using the Brain Extraction Tool (Smith, 2002) with a brain extraction factor of 0.2. FA images were created by fitting a tensor model to the raw diffusion data using FDT. Individual FA maps were visually inspected for the presence of significant residual motion or other artifacts.

All subjects' FA data were then aligned into a common space using the nonlinear registration tool FNIRT (Andersson et al., 2007a, 2007b), which employs a b-spline representation of the registration warp field (Rueckert et al., 1999). Next, the mean FA image was created and thinned to create a mean FA skeleton, which represents the centers of all tracts common to the group. Each subject's aligned FA data were then projected onto this skeleton and the resulting skeletonized, fully non-linearly aligned FA data were then used for voxelwise cross-subject statistical analysis. First, we conducted a whole brain analysis to identify possible white matter regions showing sex differences. Second, we obtained the mean FA values of each region in which the TBSS indicated statistically significant sex differences using a mask.

2.3.2. Statistical analysis

TBSS was performed on the FA maps of male and female controls. Skeletonized data were statistically analyzed using a two sample t-test. The statistical threshold was set at $p < 0.05$ FWE-corrected, using the Threshold-Free Cluster Enhancement (TFCE) method to define the clusters (Smith et al., 2006).

Statistical tests for quantified FA values and the age variable were performed with SPSS 16.0 (SPSS Inc., Chicago, Illinois). For group comparisons of FA values we used MANOVA followed by Scheffé post-hoc contrasts. The level of significance was set at $p < 0.05$.

3. Results

Whole brain TBSS analysis showed that control males have significantly higher FA values than the control female group in the anterior and posterior parts of the right superior longitudinal fasciculus, forceps minor, and the corticospinal tract (Fig. 1 and Table 2). The contrast analysis testing for females being greater than males did not show any significant difference.

We performed ANOVA analyses of the FA mean values for each region that showed sexual differences. Compared to control females, FtM showed greater FA values in the right anterior and posterior part of the superior longitudinal fasciculus (SLF), the forceps minor and the corticospinal tract (Fig. 2 and Table 3). Compared to control males, FtM showed lower FA values only in the corticospinal tract.

4. Discussion

4.1. General discussion

Measuring FA, an indicator of white matter coherence and axonal organization (Lebel et al., 2008), the main result of our study is that untreated FtM transsexuals differed from control females in two associative fasciculi (superior longitudinal fasciculus and forceps minor) and in the corticospinal tract. In contrast they only differed from control males in the corticospinal tract. These findings indicates that prior to hormonal cross-sex treatment the white matter microstructure of associative fascicles in untreated FtM transsexuals is more like that of individuals with the same gender identity than of individuals with the same biological sex.

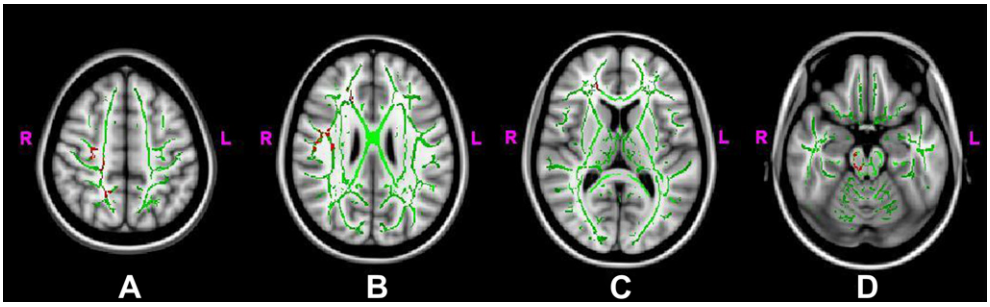

Fig. 1. Sex differences maps of fractional anisotropy (FA). FA is lower in female than in male controls in the superior longitudinal fasciculus with a posterior (A) and anterior (B) predominance. Control females also show lower than control male FA values in the forceps minor (C) and the corticospinal tract (D). The group skeleton used for the between group contrast study is green. The red color shows the clusters of significantly decreased FA in female compared to male controls. The threshold for significance was set at $p < 0.05$ corrected for multiple comparisons.

Table 2
Coordinates of the clusters showing significant differences between male and female controls.

Locations	MNI coordinates					
	x	*y*	*z*	*t*	*p*	Cluster size
Control males > Control females						
Superior longitudinal fasciculus (right, anterior)	30	−18	47	4.41	<0.01	772
Superior longitudinal fasciculus (right, posterior)	9	−42	68	4.33	<0.02	605
Forceps minor (right)	20	28	26	4.18	<0.03	524
Corticospinal tract	8	−28	−30	4.62	<0.01	705

Location *x*, *y* and *z* coordinates are based on the atlas of the Montreal Neurological Institute (MNI).

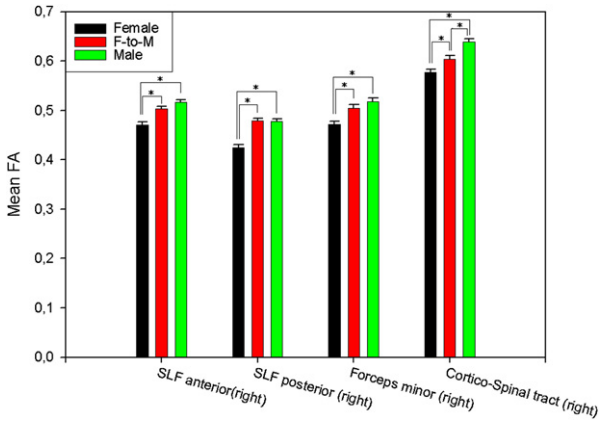

Fig. 2. Histograms showing the FA means and SD between control females (black), female to male transsexuals (FtM) (red) and control males (green). FtM transsexuals significantly differed from control females in the same white matter cerebral regions in which control males differed from control females. Significant differences between FtM transsexuals and control males are only seen in the corticospinal tract. SLF: Superior longitudinal fasciculus. *at least $p < 0.005$. (For interpretation of the references to colour in this figure legend the reader is refered to the web version of this article)

FtM transsexuals have greater FA values than control females in the white matter involving the anterior and posterior part of the SLF and the forceps minor. These fasciculi connect complex cortical regions subserving higher cognitive functions that are sexually dimorphic. Sex differences in cognition are consistently found in spatial abilities and verbal fluency (Kimura, 1999), males outshine females in the former but the females outshine males in the latter. Some studies show that males and females show comparable fMRI activation during these tasks (Carrillo et al., 2010; Halari et al., 2006), but other studies have reported differences between the sexes (Gizewski et al., 2006; Hugdahl et al., 2006; Jordan et al., 2002; Konrad et al., 2008). Cognitive studies are discordant; hormonally untreated FtM patients show spatial abilities and verbalization consistent with their biological sex and not their gender identity (Haraldsen et al., 2003). Meanwhile, others have reported that untreated MtF had higher scores on visuo-spatial tasks than untreated FtM transsexuals (Slabbekoorn et al., 1999). When verbal memory was studied, "word-correct" scores of the FtM transsexuals were lower than those of female controls, whereas scores of the MtF group were higher than those of the male controls (Cohen-Kettenis et al., 1998).

Control males and FtM present greater FA values than control females in the right forceps minor. However, no differences were seen contrasting the control male FA values with those of FtM transsexuals. The forceps minor connects, via the genu of the CC, orbitofrontal regions (Park et al., 2008) involved in emotional functions and behavioral control. Kringelbach and Roll (2004), suggested that orbital cortex activity is related to the reward value of reinforcers and punishers.

The forceps minor is a part of the anterior region of the corpus callosum. The CC is the major interhemispheric pathway in the human brain and integrates sensory, motor, cognitive and emotional functions from both hemispheres; the isthmal area of the CC is larger in homosexual than in heterosexual males (Witelson et al., 2008). FA values in the anterior part of the CC change during development (Lebel et al., 2008). In a sample of children and adolescents, it was reported that girls have lower FA values than boys in the anterior CC region (Schmithorst et al., 2008). In addition, the boys had lower FA values than girls in a small region of the splenium. We did not observe differences in the splenium between adult male and female controls.

There are only a couple of studies on the transsexual CC. Studying the CC shape, it was concluded that the shape in transsexuals is more similar to their gender identity than to their biological sex (Yokota et al., 2005). However, when the whole CC surface was studied no differences in CC -regardless of genetic sex or gender- were reported (Emory et al., 1991). Our results cannot be compared to these works, which used surface measurements, while we have used FA analysis, which seems to be a more suitable technique for detecting microstructural white matter differences.

The SLF connects anterior prefrontal and posterior parietal associative tertiary areas and develops gradually until the late twenties (Lebel et al., 2008). This fasciculus is involved in the integration of inputs from multiple modalities and is a component of the network for spatial awareness that plays a major role in the visual and oculomotor aspects of spatial function such as spatial attention and spatial working memory (Schmahmann and Pandya, 2006). Visual-spatial functions and working memory are known to have a clear sexual dimorphism (Kimura, 1999). Mental rotation and line angle judgment performance have been found to be superior in males compared to females in a worldwide study (Lippa et al., 2009). In the right SLF we found differences, male controls show greater FA values than female controls. Interestingly, untreated FtM transsexuals, like control males, also show greater FA values than control females in the right SLF and do not differ from male controls.

Table 3
Group comparisons of the fractional anisotropy in the four fasciculi that present sex differences.

	Female		FtM[a]		Male		*F*	*p*
	Mean	SD	Mean	SD	Mean	SD		
Superior longitudinal fasciculus (anterior; right)	0.47	0.022	0.50	0.024	0.52	0.021	21.16	<0.001
Superior longitudinal fasciculus (posterior; right)	0.42	0.027	0.48	0.021	0.48	0.017	24.54	<0.001
Forceps minor (right)	0.47	0.027	0.50	0.034	0.52	0.028	38.16	<0.001
Corticospinal tract (right)	0.57	0.027	0.60	0.028	0.64	0.031	12.43	<0.001

[a] Female to male transsexuals (FtM).

The corticospinal tract is a descending motor pathway originated in the precentral gyrus and in the paracentral lobule (Brodmann's areas 4 and 6; Schoenen and Grant, 2004). These motor fibers control limb movements that require a high degree of skill and flexibility. Motor experience and genetic factors critically interact during the maturation of the corticospinal tract (Cheeran et al., 2009; Martin et al., 2007). The FA values in our FtM transsexuals fell between those of the male and female control groups and differed significantly from both of them.

Mammalian brain sex differences, including humans (García-Falgueras et al., 2006), occur in complex networks (Segovia and Guillamón, 1993). The human brain differentiates early in development (Swaab, 2004). Our results indicate that two important brain associative fascicles (superior longitudinal fasciculus and forceps minor), involved in high cognitive functions, are already masculinized in FtM transsexuals before they begin cross-sex hormonal treatment.Similar conclusions have been reached by postmortem studies of the gray matter in MtF transsexuals. Hormone treatment or sex hormone levels variations in adulthood did not seem to have influenced the size and the number of neurons in the BSTc (Kruijver et al., 2000; Zhou et al., 1995) and the INAH3 (Garcia-Falgueras and Swaab, 2008). In the same way, our study suggests a prior masculinization of the white matter microstructure in FtM transsexuals.

Regarding brain laterality, we found that all the FA value decreases in women compared to men are seen in the right hemisphere. Similar asymmetries are also reported by Schmithorst et al. (2008), they described lower FA values in girls than in boys, and although there were decreases on both sides, the largest lost FA value clusters were on the right, indicating a right hemispheric predominance in sex differences. More recently Huster et al. (2009), focusing on the midcingulum bundle, found lower FA values in the right hemisphere than in the left and in women than in men.

4.2. Strengths and limitations

The current study has several strengths. It is the first to study the white matter microstructure in transsexuals. Second, the subjects have never received cross-sex hormone treatment. Third, the hormone assays preclude the presence of the polycystic ovarian syndrome as a confounding factor. Finally, the differences between FtM transsexuals and females are seen in all the regions in which males and females differed.

One limitation of this study is that the conclusions are not generalizable to MtF the transsexual subjects since we have not included a cohort of non-treated MtF transsexuals. In our population, a high percentage of MtF transsexuals start taking hormones without a physician's prescription before they contact our gender identity unit (Gómez-Gil et al., 2009a), and this precludes their inclusion in the present study.

Although we can conclude that there are *a priori* structural brain differences suggesting masculinization of untreated FtM transsexuals, these differences are not seen in the entire brain, but in specific regions of four fascicles. Moreover, we cannot exclude the possibility that their future hormonal treatment and surgical treatments could affect their brain white matter microstructure after treatment. To solve this question pre and post treatment studies or, at least, comparisons with treated groups are needed.

5. Conclusion

In conclusion, our results show that the white matter microstructure pattern in untreated FtM transsexuals is closer to the pattern of subjects who share their gender identity (males) than to those who share their biological sex (females). Our results provide evidence for structural differences in the untreated FtM transsexual's brain.

Contributors

None.

Conflict of interest

None.

Role of funding source

Funding for this study was provided by the Spanish Ministerio de Ciencia e Innovación (MNICIN) grant SEJ2007-65686 (Dr. Guillamon). MNICIN had no further role in any step of the present study.

Acknowledgments

We are grateful to the patients and control subjects that voluntarily participate in the study. Thanks are due to Drs. M. A. Amerigo, N. Bargalló, C. Falcón, J. Llul and S. Juanes for their help at some phases of the study and to R. Sánchez and C. Warren for their editorial help.

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