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Cortical Thickness in Untreated Transsexuals

Leire Zubiaurre-Elorza[1,3], Carme Junque[1,3], Esther Gómez-Gil[2,4], Santiago Segovia[5], Beatriz Carrillo[5], Giuseppina Rametti[1] and Antonio Guillamon[5]

[1]Departamento de Psiquiatría y Psicobiología Clínica, [2]Unidad de Identidad de Género, Hospital Clinic, Universidad de Barcelona, Barcelona, Spain [3]Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), Barcelona, Spain, [4]Clinical Institute of Neuroscience, Hospital Clinic i Provincial, Barcelona, Spain and [5]Departamento de Psicobiología, UNED, Madrid, Spain

Address correspondence to Antonio Guillamon, Departamento de Psicobiología, UNED, c/Juan del Rosal, 10, 28040 Madrid, Spain.
Email: aguillamon@psi.uned.es

Sex differences in cortical thickness (CTh) have been extensively investigated but as yet there are no reports on CTh in transsexuals. Our aim was to determine whether the CTh pattern in transsexuals before hormonal treatment follows their biological sex or their gender identity. We performed brain magnetic resonance imaging on 94 subjects: 24 untreated female-to-male transsexuals (FtMs), 18 untreated male-to-female transsexuals (MtFs), and 29 male and 23 female controls in a 3-T TIM-TRIO Siemens scanner. T_1-weighted images were analyzed to obtain CTh and volumetric subcortical measurements with FreeSurfer software. CTh maps showed control females have thicker cortex than control males in the frontal and parietal regions. In contrast, males have greater right putamen volume. FtMs had a similar CTh to control females and greater CTh than males in the parietal and temporal cortices. FtMs had larger right putamen than females but did not differ from males. MtFs did not differ in CTh from female controls but had greater CTh than control males in the orbitofrontal, insular, and medial occipital regions. In conclusion, FtMs showed evidence of subcortical gray matter masculinization, while MtFs showed evidence of CTh feminization. In both types of transsexuals, the differences with respect to their biological sex are located in the right hemisphere.

Keywords: cortical thickness, magnetic resonance imaging, sex differences, transsexualism

Introduction

Transsexualism is a gender identity disorder (American Psychiatric Association 2000) of unknown etiology, but it has been suggested that biological and environmental factors may contribute to gender identity variations (Cohen-Kettenis and Gooren 1999; Savic et al. 2010). Female-to-male transsexuals (FtMs) and male-to-female (MtFs) transsexuals are characterized by persistent male or female identification and uneasiness with their assigned gender; they show cross-dressing and search for hormonal and surgical sex reassignment.

Possible influences on the sexual differentiation of the brain have been used to address transsexualism in "postmortem" neuroanatomical studies (Zhou et al. 1995; Kruijver et al. 2000; Garcia-Falgueras and Swaab 2008) as well as in structural (Emory et al. 1991; Yokota et al. 2005; Luders et al. 2009; Rametti, Carrillo, Gómez-Gil, Junque, Segovia et al. 2011; Rametti, Carrillo, Gómez-Gil, Junque, Zubiarre-Elorza et al. 2011; Savic and Arver 2011) and functional positron emission tomography (Berglund et al. 2008) and magnetic resonance imaging (MRI; Gizewski et al. 2009; Schöning et al. 2010) studies.

There are very few studies on the brain structure of transsexuals. Postmortem brain studies have shown that some subcortical structures are feminized in MtFs. The volume and the number of neurons of the central part of the bed nucleus of the stria terminalis (BSTc) and the third interstitial nucleus of the anterior hypothalamus (INAH3), which present sex differences in control subjects, are feminized in MtFs (Zhou et al. 1995; Kruijver et al. 2000; Garcia-Falgueras and Swaab 2008). Moreover, the neuron number of the BSTc of an FtM was found to be in the male range (Kruijver et al. 2000). A similar pattern was reported for the volume and the number of neurons in the INAH3 of an FtM subject (Garcia-Falgueras and Swaab 2008).

Many MRI studies on brain characteristics of transsexualism have focused on white matter. Although Emory et al. (1991) found no differences for the whole corpus callosum (CC) between MtFs and FtMs and controls, Yokota et al. (2005) showed that the pattern of CC shape in both FtMs and MtFs was closer to that of individuals with the same gender identity than to individuals of the same biological sex. More recently, using the diffusion tensor imaging technique, white matter microstructure has been studied in FtMs before (Rametti, Carrillo, Gómez-Gil, Junque, Segovia et al. 2011) and after (Rametti et al. 2012) cross-sex hormonal treatment. Sex differences were reported in brain fascicules involved in higher cognitive functions. In controls, males have significantly higher fractional anisotropy (FA) values than females in the right superior longitudinal fasciculus (SLF), the forceps minor, and the corticospinal tract (CST). Compared with control females, untreated FtMs showed significantly higher FA values in the right SLF and the right forceps minor, but these FA values did not differ from those of the control males, suggesting a masculinization of these fascicles. However, the CST of FtMs had a FA value that was statistically halfway between both male and female controls, in this case suggesting an incomplete feminization or defeminization of this fascicle. Moreover, androgenization treatment increased the FA in the SLF and the CST (Rametti et al. 2012). On the other hand, MtFs showed FA values significantly halfway between male and female controls in the sexually dimorphic SLF (bilateral), right anterior cingulum, right forceps minor, and right CST; consequently, it was suggested that there was an incomplete masculinization or demasculinization in the FA pattern of these fascicles (Rametti, Carrillo, Gómez-Gil, Junque, Segovia et al. 2011).

There are a couple of voxel-based morphometry (VBM) studies on the gray matter of MtFs. Luders et al. (2009) observed that regional gray matter variation in MtFs is more similar to the pattern found in males than in females.

However, they also found that MtFs had a significantly larger volume in the right putamen than control males. Savic and Arver (2011) described a complex pattern of increases and decreases in cortical regional volumes among MtFs and male and female controls. They concluded that there is no evidence for the feminization of MtFs. There is as yet no report on the gray matter of FtMs.

Cortical thickness (CTh) is more specific than volumetric measurements to estimate between-group gray matter differences (Winkler et al. 2010). This measurement has been widely used to investigate brain sex differences. Luders et al. (2006) described greater CTh in women compared with men involving frontal, parietal, and occipital, but not temporal, lobes. Sowell et al. (2007) reported, in a large sample of subjects, thicker cortices in females than in males in the associative cortices of the temporal and parietal right hemisphere. Lv et al. (2010), in a Chinese sample, also found thicker cortices in females in the superior frontal gyrus, precentral gyrus, and postcentral gyrus in both hemispheres, and in the left superior parietal gyrus. A similar pattern and direction of the results were found in these 3 studies with and without controlling for the whole brain size. Thus, the CTh is not mediated by overall brain volume. Moreover, females have greater cortical asymmetries than males (Luders et al. 2006). To the best of our knowledge, there are no studies on the CTh in transsexuals.

Taking into account, the existence of 1) sex differences in CTh (Luders et al. 2006; Sowell et al. 2007; Lv et al. 2010), 2) the previous evidence regarding masculinization and defeminization of the white matter microstructure in FtMs before hormonal treatment (Rametti, Carrillo, Gómez-Gil, Junque, Segovia et al. 2011), and 2) the demasculinization of the white matter in the MtFs before hormonal treatment (Rametti, Carrillo, Gómez-Gil, Junque, Zubiarre-Elorza et al. 2011), we hypothesized that untreated FtMs would show a masculinized and/or defeminized CTh pattern in the cortex, while MtFs would show a feminized and/or demasculinized CTh. In consequence, in the present work, we study the CTh and the volume of subcortical structures of untreated FtMs and MtFs when compared with control males and females. This study may provide evidence on a structural cerebral basis for transsexualism.

Materials and Methods

Subjects Characteristics

Participants were 94 subjects; 24 untreated FtMs and 18 untreated MtFs consecutively enrolled from the patients consulting and diagnosed at the Gender Identity Unit (GIU) at the Hospital Clinic of Barcelona. The control groups included 29 males and 23 females recruited by advertisement. An extensive sociodemographic study of the characteristics of transsexuals from the GIU was published elsewhere (Gómez-Gil et al. 2009). All participants were right-handed. A part of the current sample of transsexuals participated in previous studies addressed to investigating the white matter microstructure; 18 FtMs have participated in Rametti, Carrillo, Gómez-Gil, Junque, Segovia et al. (2011) and 13 MtFs in Rametti, Carrillo, Gómez-Gil, Junque, Zubiarre-Elorza et al. (2011).

All transsexual subjects were diagnosed clinically according to the revised fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR; American Psychiatric Association 2000) and the 10th revision of the International Classification of Diseases (World Health Organization 1993). Transsexualism diagnoses were based on several semi-structured interviews done by 2 mental health professionals (psychiatrist and psychologist; Gómez-Gil et al. 2009). The gender identity unit ascribes to the standards of care guidelines of the World Professional Association for Transgender Health (Meyer et al. 2001).

The inclusion criteria for transsexuals were: 1) to present early-onset gender nonconformity, 2) to be erotically attracted to subjects with the same anatomical sex, 3) no history of previous hormonal treatment before the MRI scan, and 4) being assigned to the hormonal replacement therapy program. Early-onset gender nonconformity was established when, in addition to their GIU diagnosis, the patients also retrospectively fulfilled criteria A and B of the Gender Identity Disorder in Childhood (DSM-IV-TR). 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. Exclusion criteria for all participants were: 1) history of head trauma; 2) evidence of a neurological disorder or major medical condition; and 3) history of drug or alcohol abuse or dependence.

The hormonal levels of transsexuals were controlled by routine hospital testing. The analyses closest in time to the scanning session were assessed for the purpose of this study. Table 1 shows hormonal data of the as yet untreated patients. Competitive chemoluminiscent immunoassays were run for estradiol (ADVIA Centaur, Siemens; sensitivity: 10 pg/mL) and serum testosterone (Cobas, Roche; sensitivity 10 ng/dL); a sandwich type chemoluminiscent immunoassay was employed for sex hormone-binding globulin (SHBG; Cobas, Roche; sensitivity 0.4 nm/L). The free testosterone index was calculated as a percentage, [dividing testosterone (nmol/L)/SHBG (nmol/L)] × 100 (Vermeulen et al. 1999; Miller et al. 2004). All patients were carefully interviewed before MRI acquisition to ensure that they had not begun taking hormones on their own. The hormonal analyses were obtained after MRI scanning in 37 of the 42 patients. In the 5 cases in which hormonal analyses were obtained before MRI scanning, clinical endocrinology history confirmed that they had started the hormonal treatment after MRI data were collected. The mean number of days between MRI and hormonal analyses was 31.57 ± 45.66 days (range −57 to 125) for FtMs and 23.61 ± 73.77 days (range −150 to 114) for MtFs. All patients had normal hormonal levels for their biological sex (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 (Sheehan et al. 1998) to select controls without any psychiatric history. Only heterosexual controls were included in the study. Sexual orientation in controls was established by asking what partner (a man, a woman, both or neither) the participants would prefer or feel attraction to.

The participants came to the hospital for an MRI scan for this CTh measurement and the other measurements taken in the same experimental session. The whole MRI study lasted approximately half an hour during which time T_1-weighted images, diffusion tensor images, and a functional MRI study were acquired.

The work was conducted in accordance with the Declaration of Helsinki. Study approval was acquired from the Ethical Committee of the Hospital Clinic of Barcelona, and written informed consent was obtained from the subjects.

MRI Acquisition

High-resolution T_1-weighted images were acquired on a 3-T TIM TRIO scanner (Siemens, Erlangen, Germany) at the Centre de Diagnòstic per la Imatge (Hospital Clinic, Barcelona, Spain). These images were obtained using the following sequence parameters: time of repetition/time of echo = 2300/2.98 ms; time of inversion = 900 ms; acquisition matrix = 256 × 256, flip angle 9°.

MRI Analysis

The CTh analyses were performed using FreeSurfer (version 4.3.1) software (http://surfer.nmr.mgh.harvard.edu). This software was used to create a 3-dimensional cortical surface model of CTh using intensity and continuity information (Fischl and Dale 2000).

Table 1
Age of the subjects and hormonal levels

	FtMs ($n = 24$)	MtFs ($n = 18$)	Control females ($n = 23$)	Control males ($n = 29$)	F statistics (P-value)
Age (years)	26.21 ± 9.50	25.50 ± 6.91	31.09 ± 8.64	29.28 ± 6.35	[a]$F = 2.17$ (0.122) [b]$F = 3.02$ (0.06)
Hormonal levels[c]					
Serum testosterone (ng/dL)	48.25 ± 20.53	563.67 ± 196.40			
Free testosterone index (%)	3.53 ± 2.80	67.99 ± 22.89			
Sex steroid-binding globulin (nmol/L)	63.72 ± 4.21	30.82 ± 12.90			
17-β-estradiol (pg/mL)	107.78 ± 84.51	25.06 ± 20.85			

Results are expressed as mean ± standard deviation. FtMs, untreated female-to-male transsexuals; MtFs, untreated male-to-female transsexuals.
[a]Represent comparisons between FtMs and control groups.
[b]Represent comparisons between MtFs and control groups.
[c]Normal hormonal levels: Serum testosterone: Adult males (275–850 ng/dL) and females (10–80 ng/dL). Free testosterone index: Adult males (38–123%) and females (1–7%). 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).

The processing of T_1-weighted high-resolution images includes several procedures: Removal of nonbrain tissue (Segonne et al. 2004), automated Talairach transformation, intensity normalization (Sled et al. 1998), tessellation of the gray matter/white matter boundary, automated topology correction (Fischl et al. 2001; Segonne et al. 2007), and surface deformation to detect gray matter/white matter and gray matter/cerebrospinal fluid boundaries (Fischl and Dale 2000). Moreover, the cerebral cortex was divided into different regions according to gyral and sulcal structure information (Desikan et al. 2006). The resulting representation of CTh is calculated as the distance between tissue boundaries (gray matter/white matter and gray matter/cerebrospinal fluid; Fischl and Dale 2000). All surface models in our study were visually inspected for accuracy.

Cortical maps were analyzed at the vertex-wise level by means of a CTh general linear model approach (implemented in QDEC from FreeSurfer). Then, individual CTh maps were registered bilaterally to the standard template and smoothed with a Gaussian kernel of 15-mm FWHM. T-test comparisons were performed to evaluate CTh differences: 1) female controls versus male controls; 2) FtMs versus male controls; 3) FtMs versus female controls; 4) MtFs versus male controls; and 5) MtFs versus female controls. Family-wise error correction $P < 0.05$ with the Monte Carlo Null-Z simulation was applied to CTh maps using 10 000 permutations.

We also obtained the volumes of subcortical gray matter structures (thalamus, caudate, putamen, pallidum, hippocampus, and amygdala) and the intracranial volume by means of FreeSurfer software, then analyzing the data by SPSS v. 18.0 (SPSS Inc., Chicago, IL, United States of America). We created a new variable where subcortical volumes were corrected by intracranial volume. We performed a multivariate ANOVA with the aim of testing subcortical volume differences in each group of transsexuals (MtFs and FtMs) independently compared with both groups of controls (females and males). The Tukey statistical test was used for post hoc analyses.

Results

Cortical Thickness Analysis

Whole-brain comparison maps of CTh between male and female controls showed that females have a thicker cortex than males in several regions. We observed 3 clusters that achieved statistical significance in the inferior parietal, postcentral, and parstriangularis regions (Table 2). In the right hemisphere, females have a thicker cortex than males in the parietal and frontal regions, while in the left lobe the female's cortex is thicker in the parieto-occipital regions (Fig. 1). Males did not show any region of thicker cortex than females.

The CTh of FtMs did not differ from female controls. With respect to control males, FtMs showed the thicker cortex in the right parietal lobe and the medial left parieto-temporal regions (Fig. 1).

Table 2
Regional differences in CTh between groups

Anatomical brain regions included in a cluster	Side	Cluster size	Cluster maxima Talairach coordinates			P-value
			x	y	z	
Female controls versus male controls						
Inferior parietal, superior parietal, lateral occipital, cuneus	Left	2811.03	−31.7	−71.1	22.5	0.000
Postcentral, inferior parietal, superior parietal, precentral, supramarginal	Right	1847.97	36.9	−29.8	47.5	0.013
Parstriangularis, parsorbitalis, rostral middle frontal, frontal pole, superior frontal, medial orbitofrontal, lateral orbitofrontal	Right	3289.4	46.8	35.5	−5.1	0.000
Female-to-male transsexuals versus male controls						
Supramarginal, inferior parietal, superior temporal, banks of the superior temporal sulcus	Left	1465.54	−53.1	−50.9	23.0	0.047
Inferior parietal, postcentral, supramarginal, superior parietal	Right	1661.62	34.3	−54.7	41.1	0.026
Male-to-female transsexuals versus male controls						
Rostral middle frontal, pars triangularis, pars orbitalis, lateral orbitofrontal	Right	1414.61	39.1	39.7	2.0	0.048
Cuneus, pericalcarine, lingual	Right	1651.22	4.2	−71.2	20.3	0.019
Medial orbitofrontal, superior frontal, rostral anterior cingulate, rostral middle frontal, frontal pole	Right	1441.16	8.2	58.9	−4.0	0.044

Regions in bold represent the maximum coordinate encompassed in a given cluster.
Talairach coordinates indicate: x increases from left (−) to right (+); and y increases from posterior (−) to anterior (+); and z increases from inferior (−) to superior (+).

The analysis of CTh in the MtFs showed that they did not differ from control females. In relation to control males, MtFs have greater CTh in the right hemisphere involving the lateral and medial orbito-frontal regions, the insula, and the medial occipital cortex (Fig. 1).

Subcortical Volumetric Analysis

The volumetric analyses of subcortical gray matter structures of the FtMs and male and female control groups showed a group effect for the right putamen ($F_{2,73} = 4.11$; $P = 0.02$). Post hoc analyses showed males had greater right putamen volume than females ($P = 0.04$; Fig. 2). Moreover, the right putamen of FtMs differed in a statistically significant manner from the female control group ($P = 0.03$; Fig. 2), but they did not differ from control males.

The volumetric analysis of MtFs and male and female controls also showed group differences in the right putamen

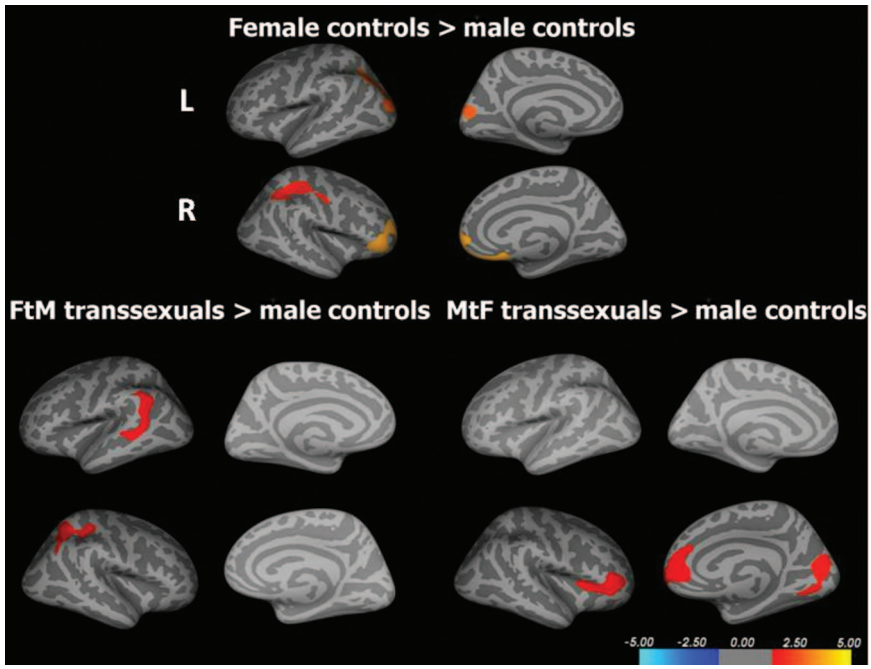

Figure 1. Maps showing statistically significant differences in CTh. The top part of the figure displays the regions in which female controls had thicker cortex than male controls. The bottom left of the figure exhibits the cortical regions in which FtMs showed thicker cortex than male controls. The bottom right shows the thicker cortex in MtFs than male controls. Color bars represent a scale of *t* values with cold colors representing thinning and warm colors thickening. FtMs, female-to-male transsexuals; MtFs, male-to-female transsexuals.

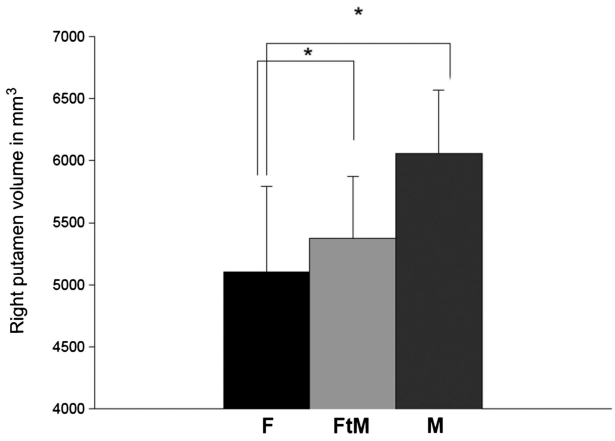

Figure 2. Histogram showing the raw mean data of the right putamen of FtMs and male and female controls. Male controls have a larger volume than female controls in the right putamen nucleus. FtMs have larger volume than female controls. F, female controls; FtMs, female-to-male transsexuals; M, male controls; $^*P < 0.05$.

($F_{2,67} = 3.62$; $P = 0.03$). Post hoc analyses showed that this effect emerged from the contrast between male and female controls ($P = 0.04$). MtFs did not differ in a statistically significant manner from either the male or the female controls. MtFs and FtMs did not show differences when compared with controls in other subcortical gray matter structures.

Discussion

We have found that control females have greater CTh compared with control males in the frontal and parietal regions; in contrast, males have a larger putamen volume than females. With respect to the transsexual groups, we observed that FtMs have greater CTh compared with control males in the parietal and temporal cortices and did not differ from control females. However, FtMs have a larger right putamen than female controls. On the other hand, MtFs did not differ from control females in CTh and had greater CTh than control males in the frontal and occipital regions. In this group, no differences were found in the putamen. All these findings suggest that FtMs have a defeminized putamen, while MtFs have a feminized CTh.

Cortical and Subcortical Sex Differences

Although CTh has not previously been reported in transsexuals, it has been widely investigated in studies focused on brain sex differences (Im et al. 2006; Luders et al. 2006; Sowell et al. 2007; Raznahan et al. 2010). All the previously published studies described a pattern of regionally thicker cortex in females compared with males over large cortical regions. The cortex of the males was only found to be significantly thicker than the females in small areas of the anterior temporal and orbitofrontal regions in the right hemisphere (Sowell et al. 2007) and anterior temporal cortex (Raznahan et al. 2010). Our results in control groups agree with the main findings of previous works, and we also found that regions of the frontal, parietal, and occipital lobes were thicker in females than in males. No regions of increased thickness were observed in males.

The subcortical gray matter volumetric analyses showed statistically significant group differences in the right putamen. Males had a larger volume than females. The basal ganglia are involved in the control of movement and in cognitive and affective behaviors (Postuma and Dagher 2006) and are sexually dimorphic, males showing larger volume in the globus pallidus (Giedd et al. 1996; Garcia-Falgueras et al. 2006;

Rijpkema et al. 2012) and the putamen (Giedd et al. 1996; Rijpkema et al. 2012). Moreover, sex differences were reported in the development of the putamen, since the putamen decreases with age in males only (Giedd et al. 1996). In addition, the volume of the putamen is larger in familial male precocious puberty (Mueller et al. 2011), thereby suggesting an early effect of androgens on the size of this structure.

Female-to-Male Transsexuals

We did not find significant differences between FtMs and female controls in CTh. This result suggests that FtMs have a CTh pattern that corresponds to their biological sex. We found the thicker cortex in FtMs than in control males in the right parietal cortex, a region in which females differed from control males. In addition, FtMs showed greater CTh in the medial left parieto-temporal cortex. Contrarily to the control females, FtMs did not differ from the control males in the prefrontal orbital regions. Thus, although we have not found signs of CTh masculinization, the pattern of differences between FtMs and control males is different from that seen between male and female controls.

In contrast to the CTh results, FtMs showed a masculinization of their right putamen, because the volume of this structure statistically differed from female but not from male controls, thus verifying our hypothesis that the gray matter of FtMs may show signs of masculinization. This is consistent with previous findings that reported brain masculinization in FtMs. In a previous study, we have found that white matter microstructure of FtMs is masculinized and defeminized (Rametti, Carrillo, Gómez-Gil, Junque, Segovia et al. 2011). The right superior longitudinal fascicle and right forceps minor are sexually dimorphic, and these fascicles are masculinized (they did not differ from control males) in FtMs, while the CST, which is also sexually dimorphic, shows defeminization (FA values were significantly halfway between male and female controls).

Male-to-Female Transsexuals

MtFs have a feminized CTh. It should be underscored that MtFs did not differ from control females in any region. In addition, they showed greater CTh than control males in the right orbitofrontal and insular cortices and in the right medial occipital region. These results verify our hypothesis regarding CTh of MtFs and confirm that their cortical gray matter is feminized. A right hemisphere demasculinization has also been observed in white matter microstructure. MtFs showed lower FA values in the SLF, the anterior cingulum, the forceps minor, and the CST (Rametti, Carrillo, Gómez-Gil, Junque, Segovia et al. 2011).

The cortical regions feminized in MtFs are involved in complex brain functions. The orbitofrontal cortex is related to emotional and social decision-making (Bechara et al. 2000), the insula serves sensory, autonomic, emotional, and cognitive functions (Shelley and Trimble 2004), and the medial occipital cortex holds visuoperceptual processes (Haxby 2006).

There are only 2 studies focused on cortical gray matter characteristics in MtFs. Luders et al. (2009) found that the cortical volumes of MtFs were similar to those of their biological sex (male). Savic and Arver (2011) reported that MtFs had a greater gray matter volume than males and females in the superior temporal and angularis gyrus. Moreover, MtFs had increased gray matter compared with males in the lingual gyrus, cerebellum, and insular cortex, and compared with control females in the insular cortex. Decreased cortical volumes in MtFs with respect to females were seen in the pre- and postcentral gyrus. They concluded that their data do not support feminization of the MtF brain. This conclusion might emerge from the fact that in the region (superior temporal + angularis) in which MtFs had a greater volume than males, no sex differences were found between control subjects. However, despite the methodological differences in the gray matter measurements and the fact that they studied MtFs who were sexually attracted to females, our results reflect the same tendency as those reported by Savic and Arver (2011). In our study, MtFs showed greater CTh than males in the orbitofrontal, insular, and medial occipital right hemisphere cortices.

The Luders et al. (2009) study is not directly comparable with ours, because it was performed using VBM. The cortical volume measures obtained by VBM procedures mix the effects of CTh with cortical surface and folding. Moreover, CTh and surface are genetically and phenotypically different and can be differentially affected by environmental factors (Panizzon et al. 2009; Winkler et al. 2010; Raznahan et al. 2011).

Postmortem studies of hormonally treated MtFs have shown that their BSTc and INAH3, which are located in regions related to sexual behavior in mammals (De Jonge et al. 1989; Claro et al. 1995), are feminized (Zhou et al. 1995; Kruijver et al. 2000; Garcia-Falgueras and Swaab 2008). In our study, the volume of the putamen of untreated MtFs did not differ significantly from either the male or female controls, although it felt halfway between the 2 volumes. There are 2 previous studies of the putamen in untreated MtFs. Luders et al. (2009), in a whole-brain VBM study, found a larger right putamen in female than male controls, and MtFs had the largest right putamen of all. In contrast, Savic and Arver (2011), using a volumetric analysis similar to ours, did not find sex differences although they reported a smaller putamen volume in MtFs with respect to male and female controls. The different analytic techniques used make it difficult to explain the results reported to date on the putamen.

On the basis of chromosomal sex and behavior, Blanchard and co-workers (Blanchard et al. 1987, 1989, 1996; Blanchard 1989; see also Smith et al. 2005) have proposed the existence of 2 types of MtFs: 1) MtFs that are attracted to males ("homosexual" transsexuals in Blanchard terminology), and 2) MtFs that are attracted to women ("heterosexual" transsexuals according to Blanchard). Further, Blanchard (2008) hypothesized that homosexual MtFs would differ from heterosexual males in brain sexually dimorphic structures, while in the heterosexual MtFs, the differences might not implicate sexually dimorphic structures. More recently, Cantor (2011) has noted that our findings on the white matter microstructure of (homosexual) MtFs (Rametti, Carrillo, Gómez-Gil, Junque, Zubiarre-Elorza et al. 2011) and that of Savic and Arver (2011) on the cortical volume of (heterosexual) MtFs would support Blanchard's hypothesis. In the present report, we studied MtF transsexuals erotically attracted to males that show a feminization of CTh but not in the putamen. Moreover, these findings on the CTh show the same tendency as those reported by Savic and Arver (2011) with respect to the cortical volume of MtFs erotically attracted to females. Consequently, to verify

Blanchard's hypothesis would require a specific design that is beyond the scope of the present study.

General Comments

MRI CTh reflects cellular characteristics such as number and size of cortical cells, including their packing density and also the degree of myelinization (Kruggel et al. 2003; Eickhoff et al. 2005). Recently, it has been reported that sex differences in cortical structure vary by cortical region and developmental stage. Raznahan et al. (2010) reported that, in 9-year-old children, the mean CTh of males was greater than females throughout most of the cortical mantle. Nevertheless, at 22 year old, this difference became diminished or inverted in some cortical subregions and accentuated in others. The progressive thinning of the cortex has been related to pruning (Huttenlocher 1990). The cortical thinning is mediated by the efficiency of androgen receptors in both males and females (Raznahan et al. 2010).

Probably, the developmental focus is the best to understand our results. Brain development from childhood to adulthood is characterized by decreases in gray matter (Giedd et al. 1996; Reiss et al. 1996; Shaw et al. 2008; Raznahan et al. 2010) that follows a heterogeneous dynamic (Shaw et al. 2008; Østby et al. 2009; Raznahan et al. 2010) that is determined by regional genetic (Rimol et al. 2010) and hormonal (Raznahan et al. 2010) variables. The result of this process is that males show a thinner cortex than females (Raznahan et al. 2010), and it should be highlighted that the efficiency of the androgen receptor plays an important role in cortical thinning (Raznahan et al. 2010).

The CTh of FtMs seems to follow the expected pattern of female cortical development, since it did not differ from females and shows a thicker temporo-parietal region than in control males, suggesting FtMs have followed a female cortical thinning pattern of development. This region is involved in the visuospatial abilities that are sexually dimorphic. Females and FtMs before androgenization treatment do not perform as well as males on visuospatial tasks (Voyer et al. 1995; Haraldsen et al. 2003).

With respect to the CTh of MtFs, we found that this group did not differ from female controls but did from male controls. These findings suggest that MtFs follow the pattern of cortical thinning typically described for females. Whether the cortical feminization of MtFs depends on a differential cortical androgen receptor distribution, a different efficiency in the androgen receptors or other causes remains to be elucidated. But what seems clear is that in MtFs the cortical developmental process is affected and follows the direction expected for females. This points out that the developmental approach could help to understand the etiology of transsexualism.

Related to subcortical gray matter findings, it should be noted that the putamen is a structure that presents a linear decrease in volume from childhood to adulthood (Østby et al. 2009), and developmental curves show the largest volumes in males (Giedd et al. 1996; Rijpkema et al. 2012). In the case of FtMs, the right putamen seems to have followed a masculinized developmental pattern. FtMs have a larger volume of this structure compared with control females, but they did not significantly differ from males.

Considering the present work on cortical and subcortical gray matter and the data available in the literature reviewed above, what can we say of the brain of transsexuals? We would suggest that transsexuals do not show a simple masculinization (FtMs) or feminization (MtFs) of their brains—rather, they present a complex picture in their process of sexual differentiation depending on the brain region studied and the kind of measurements taken. Untreated FtMs show patterns of masculinization and defeminization of their white matter (Yokota et al. 2005; Rametti, Carrillo, Gómez-Gil, Junque, Segovia et al. 2011), while keeping a female CTh that differs from males in a peculiar manner but shows a masculinized putamen volume. On the other hand, MtFs show a pattern of feminization and demasculinization of their white matter (Yokota et al. 2005; Rametti, Carrillo, Gómez-Gil, Junque, Zubiarre-Elorza et al. 2011) and feminization of their CTh but no clear effects on their cortical volume (Luders et al. 2009; Savic and Arver 2011).

These considerations as a whole and our present results provide, for the first time, some clues as to what could developmentally happen in the brain differentiation process of transsexuals: FtMs show a subcortical masculinization, while MtFs predominantly show a feminization of cortical regions. In both types of transsexuals, the pattern of differences with respect to their biological sex is located in regions of the right hemisphere. This might suggest a hemispheric asymmetry in the effect of androgens during the development of the brain in transsexuals.

Limitations and Strengths

One limitation of our sample is that we did not assess the menstrual cycle phase in FtMs and control women. The menstrual cycle produces changes in the volume of the amygdala and hippocampus (Protoppopescu et al. 2008; Ossewaarde et al. 2011). Nevertheless, to the best of our knowledge, there is no report in the literature on the effect of the menstrual cycle on CTh and the volume of the putamen.

We feel the strengths of this particular investigation stem from the use of a homogeneous sample. Both MtFs and FtMs were early onset and erotically attracted to the same anatomical sex. This type of transsexual is the most representative of the general population of transsexuals (Gómez-Gil et al. 2009; Lawrence 2010). Moreover, to the best of our knowledge, this is the first report on CTh of this kind of transsexuals.

Notes

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