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Effects of Cross-Sex Hormone Treatment on Cortical Thickness in Transsexual Individuals

Leire Zubiaurre-Elorza, PhD,*† Carme Junque, PhD,*† Esther Gómez-Gil, MD, PhD,‡§ and
Antonio Guillamon, MD, PhD¶

*Departamento de Psiquiatría y Psicobiología Clínica, Universidad de Barcelona, Barcelona, Spain; †Institute of
Biomedical Research August Pi i Sunyer (IDIBAPS), Barcelona, Spain; ‡Unidad de Identidad de Género, Hospital Clínic,
Universidad de Barcelona, Barcelona, Spain; §Clinical Institute of Neuroscience, Hospital Clinic i Provincial, Barcelona,
Spain; ¶Departamento de Psicobiología, UNED, Madrid, Spain

A B S T R A C T

Introduction. Untreated transsexuals have a brain cortical phenotype. Cross-sex hormone treatments are used to masculinize or feminize the bodies of female-to-male (FtMs) or male-to-female (MtFs) transsexuals, respectively.
Aim. A longitudinal design was conducted to investigate the effects of treatments on brain cortical thickness (CTh) of FtMs and MtFs.
Methods. This study investigated 15 female-to-male (FtMs) and 14 male-to-female (MtFs) transsexuals prior and during at least six months of cross-sex hormone therapy treatment. Brain MRI imaging was performed in a 3-Tesla TIM-TRIO Siemens scanner. T1-weighted images were analyzed with FreeSurfer software to obtain CTh as well as subcortical volumetric values.
Main Outcome Measures. Changes in brain CTh thickness and volumetry associated to changes in hormonal levels due to cross-sex hormone therapy.
Results. After testosterone treatment, FtMs showed increases of CTh bilaterally in the postcentral gyrus and unilaterally in the inferior parietal, lingual, pericalcarine, and supramarginal areas of the left hemisphere and the rostral middle frontal and the cuneus region of the right hemisphere. There was a significant positive correlation between the serum testosterone and free testosterone index changes and CTh changes in parieto-temporo-occipital regions. In contrast, MtFs, after estrogens and antiandrogens treatment, showed a general decrease in CTh and subcortical volumetric measures and an increase in the volume of the ventricles.
Conclusions. Testosterone therapy increases CTh in FtMs. Thickening in cortical regions is associated to changes in testosterone levels. Estrogens and antiandrogens therapy in MtFs is associated to a decrease in the CTh that consequently induces an enlargement of the ventricular system. **Zubiaurre-Elorza L, Junque C, Gómez-Gil E, and Guillamon A. Effects of cross-sex hormone treatment on cortical thickness in transsexual individuals. J Sex Med **;**:**–**.**

Key Words. Transsexuals; Cortical Thickness; MRI; Cross-Sex Hormone Therapy; Testosterone; Estrogens; Antiandrogens; Anabolic Steroids; Gender Dysphoria; Gender Identity Disorders; Sex Steroid Hormone Therapy

Introduction

Transsexuals (female-to-male [FtMs] and male-to-female [MtFs]) are characterized by persistent cross-sex identification and uneasiness with their assigned sex. They consequently desire and search out cross-sex hormonal treatment and surgical sex reassignment.

Recently, transsexual cerebral phenotypes have been described before cross-sex hormonal treatment [1–6]. Untreated FtMs had a similar cortical thickness (CTh) to control females and greater

CTh than males in the parietal and temporal cortices. With respect to subcortical structures, FtMs have a larger right putamen than females but do not differ from control males [6]. On the other hand, the CTh in untreated MtFs did not differ from that in female controls but was greater than in control males in the orbitofrontal, insular and medial occipital regions, a greater size in all these areas reflecting CTh feminization [6]. It was also reported that MtFs have greater CTh than control males [2]. Moreover, voxel-based studies of cortical volumetric measures run in the same direction [5]. Finally, most of the differences shown in untreated FtMs and MtFs are in the right hemisphere [3,6].

Sex steroid hormones have a wide and varied effect on body tissues. In the brain they are involved in sexual differentiation, development and behavior. In gray matter, androgen receptors (ARs) [7–9] as well as α and β-estrogen receptors (ERs) [10–12] have been observed in primate and human cortices. In subcortical structures, ARs [13] and ERs have also been detected in humans [14,15]. In addition, ARs have been identified in axons, dendrites and glial cells in the rat cortex and amygdala [16] as well as in astrocytes and oligodendrocytes in the primate prefrontal cortex [8].

To the best of our knowledge only two published studies have focused on the effect of cross-sex treatments on the brain of transsexuals. Hulshoff Pol et al. [17] found that testosterone treatment increased total brain and hypothalamus volumes in FtMs while treatment with estrogens and antiandrogens decreased brain volumes of MtFs. Recently, Rametti et al. [18] reported that testosterone treatment increased fractional anisotropy (FA) values in the right superior longitudinal fasciculus and the right corticospinal tract of FtMs. These increments could be predicted from the free testosterone index before the hormonal treatment.

Cross-sex hormonal treatment with testosterone is directed toward body masculinization in FtMs whereas estrogens and antiandrogens are administered to produce a feminine body in MtFs [19,20]. Therefore, given that: (a) the gray matter contains AR and ER [7,9,11–14]; (b) testosterone treatment increases the volume of the hypothalamus in FtMs [17] and FA values of sexually dimorphic fascicles [18] while (c) estrogens + antiandrogens decreases brain volumes in MtFs [17]; the aim of the present study was to explore the effects of cross-sex hormonal treatment on the CTh of FtMs and MtFs. With the paucity of the available literature, only a broad exploratory hypothesis could be advanced: we expected that hormonal treatments would modify CTh and the volumes of subcortical structures in our subjects. To address this possibility we performed a pre-post treatment study that independently compared the CTh of FtMs and MtFs before and after cross-sex hormonal treatment. This provides a unique opportunity to investigate the effects of cross-sex hormone treatment on the brain in humans.

Methods

Participants

Written informed consent was obtained from 15 FtMs and 14 MtFs patients treated at the Gender Identity Unit (GIU) at the Hospital Clínic of Barcelona (Spain). The current sample shows similar social and demographic characteristics previously described for Spanish transsexuals [21]. All participants were right-handed.

All transsexual subjects were diagnosed clinically according to the revised fourth edition of the Diagnostic and Statistical Manual of Mental Disorders [22] and the tenth revision of the International Classification of Diseases [23]. Transsexualism diagnoses were based on several semi-structured interviews done independently by a psychiatrist and a psychologist [21]. The GIU ascribes to the standards of care guidelines of the World Professional Association for Transgender Health (WPATH) [24]. All subjects selected for this study evidenced early-onset gender nonconformity, were erotically attracted to females (FtMs) or males (MtFs), began cross-sex hormonal treatment, and were interested in sex reassignment. The inclusion criteria were: (1) no history of previous hormonal treatment before the first MRI scan and (2) to have been receiving cross-sex hormonal treatment for at least six months at the time of the second scan. 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 first MRI scan was performed before beginning hormonal treatment and the second MRI was obtained after at least 6 months of hormonal treatment (Table 1). At the time of the second scan no subject had yet undergone mastectomy, hysterectomy, and/or phaloplasty (FtMs) and orchidectomy or vaginoplastia (MtFs). The work was conducted in accordance with the Declaration of Helsinki. Study approval was acquired

Table 1 Characteristics of the sample and hormone levels

	FtM[†] Pretreatment	FtM Posttreatment[¶]	t statistics (*P* values)
Age (years)	24.93 ± 7.37[§]	26 ± 7.73	5.17 (0.001)
Months of treatment		9.80 ± 2.04 (8–16)	
Hormonal levels[§]			
Testosterone (ng/dL)	51.84 ± 20.12	749.17 ± 257.96	10.34 (0.001)
Testosterone index (%)	4.21 ± 3.15	11.95 ± 63.10	6.82 (0.001)
Sex steroid binding globulin (nmol/L)	55.77 ± 27.38	29.07 ± 15.48	5.94 (0.001)
	MtF[‡] Pretreatment	MtF Posttreatment[††]	
Age (years)	24.64 ± 7.62	25.86 ± 7.79	7.85 (<0.001)
Months of treatment, range (min-max)		8.00 ± 4.47 (6–30)	
Hormonal levels[‡‡]			
Testosterone (ng/dL)	548.15 ± 188.90	23.51 ± 16.25	9.91 (<0.001)
Testosterone index (%)	64.11 ± 21.11	2.40 ± 3.32	10.31 (<0.001)
Sex steroid binding globulin (nmol/L)	31.02 ± 10.03	49.41 ± 24.56	3.47 (0.004)
17-b-estradiol (pg/mL)	26.29 ± 24.08	47.14 ± 42.48	1.91 (0.078)

[†]FtM = female-to-male transsexuals
[‡]MtF = male-to-female transsexuals
[§]Mean ± standard deviation
[¶]treatment: testosterone
[††]treatment: estrogens + antiandrogens
[‡‡]Normal hormonal levels: *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); *estradiol*: males (10–41 pg/mL); females: follicular phase (22–55 pg/mL), luteal phase (68–196 pg/mL)

from the Ethical Committee of the Hospital Clínic of Barcelona.

Hormone Treatment and Measurements

FtMs received either intramuscular depot injections of testosterone undecanoate (500 to 1,000 mg) every 10–14 weeks or transdermal testosterone gel (50 mg every 24–48 hours). The range of treatment time for these subjects before the second scan was 8 to 16 months. MtFs received treatment with estrogens: estradiol valerate tablets (2–4 mg/day, eleven of the patients), conjugated estrogen tablets (2.5 mg/day, one patient) or transdermal 17 β-estradiol patches (6 mg/day, two patients). Since none of them have undergone vaginoplasty at the time of the study, the antiandrogen cyproterone acetate tablets (25 to 50 mg/day) were associated to estradiol in all patients. The duration of the treatment before the second scan ranged from 6 to 30 months. The hormone administration route was decided by the patient's personal preferences.

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). 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) by SHBG (nmol/L) × 100 [25,26].

MRI Acquisition

High-resolution T1-weighted images were acquired using a 3-Tesla TIM TRIO scanner (Siemens, Erlangen, Germany) at the *Centre de Diagnòstic per la Imatge* (Hospital Clínic, Barcelona, Spain). These images were obtained using the following sequence parameters: TR/TE = 2300/2.98 ms; TI = 900 ms; acquisition matrix = 256 × 256, flip angle 9° with a voxel size of 1 × 1 × 1 mm.

MRI Analyses

For all procedures described below FtMs and MtFs were processed separately. To extract reliable volume and thickness estimates between the two time points (before and after hormonal treatment) images were automatically processed with the longitudinal stream [27] in FreeSurfer (version 5.1) software (http://surfer.nmr.mgh.harvard.edu). This method creates an unbiased within-subject template space and image [28] using robust, inverse consistent registration [29]. Processing steps (skull stripping, Talairach transforms, atlas

Table 2 Global ventricular system volume before and after cross-sex hormonal treatment

	Pretreatment	Posttreatment	t statistics (*P* values)	Effect size[†]
Female-to-male	12.09 ± 4.02[‡]	12.17 ± 4.10[§]	t = −0.37 (0.720)	0.02
Male-to-female	16.63 ± 7.94	18.81 ± 8.35[¶]	t = −5.88 (<0.001)	0.27

All values are given in cm[3].
[†]Effect size: 0.20 (small); 0.20-0.50 (medium) and >0.50 (large)
[‡]Mean ± SD
[§]treatment: testosterone
[¶]treatment: estrogens + antiandrogens

registration, spherical surface maps and parcellations) were then initialized with common information from the within-subject template, significantly increasing statistical power and reliability [27]. Moreover, the cerebral cortex was segmented into 34 different gyral regions per hemisphere (13 frontal, 9 temporal, 7 parietal, 4 occipital, and insula) using the Desikan-Killiany Atlas [30]. For each of these regions mean CTh was calculated as the distance (in mm) between tissue boundaries (gray matter/white matter and gray matter/cerebrospinal fluid) [31]. Procedures for the measurement of CTh have been validated against manual measurements [32,33] and histological analysis [34]. All surface models in our study were visually inspected for accuracy.

For subcortical structures, we also obtained the volumes of these regions and the intracranial volume by means of Free-Surfer software and the data were analyzed by SPSS software. We performed a multivariate ANOVA with intracranial volume as covariate with the aim of testing subcortical volume differences between both time points (pre- and posttreatment).

Statistical Analyses
Statistical analyses were carried out using SPSS version 18.0 (SPSS Inc., Chicago, IL). The data were tested for normality and homogeneity. To test the differences of hormone levels paired t-test was used. The effect size index was also calculated [35] to determine the degree to which our results have practical significance in the transsexual population.

Surface analyses were then performed using QDEC toolbox of FreeSurfer (http://www.surfer.nmr.mgh.harvvard.edu). By means of a CTh general linear model approach CTh bilateral maps were analyzed at the vertex-wise level after registered to the standard template and smoothed with a Gaussian kernel of 15 mm FWHM.

A measure of the change rate (mm of CTh/year) was used to perform correlation analyses with hormonal indices (serum testosterone, free testosterone index and estradiol). Family wise error-correction with Monte Carlo Null-Z simulation was applied to CTh maps with 10,000 permutations. Next, by using SPSS software a paired t-test was performed in order to determine whether the regional CTh and cortical and subcortical volumes differed between the two time points.

Results

Female-to-Male Transsexuals
Brain Volumes
The total (cortical and subcortical) gray matter volume increased after testosterone treatment (t = 2.74 9; *P* < 0.01). Before treatment the mean total volume was 637.74 ± 31.9 cm^3, and after treatment the mean was 647.44 ± 29.21 cm^3. Cortical gray matter volume also differed before (458.72 ± 28.13 cm^3) and after treatment (466.75 ± 25.69 cm^3) t = 2.79, *P* < 0.01. Regarding the volumes of the gray matter subcortical structures, we only detected a significant increase in the right thalamus (before treatment: 72.65 ± 5.55 cm^3; after testosterone treatment: 74.89 ± 5.98 cm^3; t = 2.630; *P* < 0.02). No effects were seen on the ventricular system (Table 2).

Cortical Thickness
After testosterone treatment, CTh increases in several cortical regions at *P* = < 0.01 (Table 3). Increases in CTh was seen bilaterally in the postcentral gyrus, and unilaterally in the inferior parietal, lingual, pericalcarine, and supramarginal regions on the left side, as well in the cuneus and rostral middle frontal areas in the right side. The effect size estimation indicates a large effect for the left lingual cortex and moderate effects in the left postcentral gyrus and the right rostral middle frontal region.

Correlation Analysis Between Cortical Thickness and Testosterone Levels
As was expected, the serum testosterone levels and free testosterone index significantly increased in

Table 3 Cortical regions showing increase in the cortical thickness of female-to-male transsexuals after androgenization

	Pre-androgenization	Androgenized	t statistics (*P* values)	Effect size[†]
Left inferior parietal	2.62 ± 0.170[‡]	2.68 ± 0.154	t = −2.88 (0.012)	0.35
Left lingual	1.99 ± 0.100	2.06 ± 0.105	t = −3.75 (0.002)	0.70
Left pericalcarine	1.61 ± 0.139	1.65 ± 0.141	t = −2.86 (0.013)	0.29
Left postcentral	2.17 ± 0.100	2.22 ± 0.090	t = −3.03 (0.009)	0.50
Left supramarginal	2.68 ± 0.132	2.73 ± 0.133	t = −2.99 (0.010)	0.38
Right cuneus	1.95 ± 0.153	2.01 ± 0.160	t = −3.36 (0.005)	0.39
Right postcentral	2.16 ± 0.119	2.20 ± 0.096	t = −3.20 (0.006)	0.34
Right rostral middle frontal	2.22 ± 0.125	2.27 ± 0.153	t = −3.14 (0.007)	0.40

[†]Effect size: 0.20 (small); 0.20-0.50 (medium); >0.50 (large)
[‡]Mean ± SD

FtMs after hormonal treatment (Table 1). Serum testosterone increased 1445% and free testosterone index 283%.

The correlation analyses between the rate of changes in CTh and the rate of changes in these hormonal measurements achieved statistical significance in the left hemisphere for serum testosterone [cluster size: 1424; Talairach coordinates (x = −45.7, y = −76.7, z = 8.3); *P* value = 0.02] in the lateral occipital, inferior parietal and fusiform areas and for the free testosterone index [cluster size: 2858; Talairach coordinates (x = −29.8, y = −88.8, z = 14.1); *P* value = 0.0001] in the lateral occipital, inferior and superior parietal and fusiform area (Figure 1).

Male-to-Female Transsexuals
Brain Volumes
Volumetric analyses showed a significant decrease in the total gray matter (pretreatment: 705.28 ± 53.87 cm³; posttreatment: 685.69 ± 50.87 cm³, t = 4.60 *P* < 0.0001). The cortical gray matter volume showed a decrease (pretreatment: 507.37 ± 36.09 cm³; posttreatment: 494.45 ± 35.90 cm³,

t = 3.34; *P* = 0.005). Subcortical gray mater volume also showed a decrease (pretreatment: 197.92 cm³ ± 20.24 cm³; posttreatment: 191.24 ± 18.57 cm³; t = 5.04; *P* < 0.0001). The subcortical decrease mainly arose from the right thalamus (pretreatment: 7.68 ± 0.67 cm³; posttreatment: 7.44 ± 0.58 cm³; t = 3.47; *P* = 0.004) and the right pallidum (pretreatment: 1.67 ± 0.15 cm³; posttreatment: 1.60 ± 0.13 cm³; t = 3.16; *P* = 0.008). In addition, we also found a statistically significant increase in the whole ventricular system (Table 2).

Cortical Thickness
The cortical regions that achieved significance at the threshold level of *P* < 0.01 are shown in Table 4. We can see that there is a significant decrease of CTh that involves mainly the cortex of the occipital, temporal and parietal regions and some areas of the frontal lobes.

Correlation Analyses Between Cortical Thickness and Hormonal Levels
As was expected, the serum testosterone levels and free testosterone index significantly decreased in

Table 4 Cortical regions showing decrease after estrogens and anti-androgens treatment in male-to-female transsexuals

	Pretreatment	Posttreatment	t statistics (*P* values)	Effect size[‡]
Left isthmus cingulate	2.79 ± 0.24[†]	2.68 ± 0.21	t = 2.97 (0.01)	0.45
Left rostral anterior cingulate	3.09 ± 0.19	2.98 ± 0.17	t = 2.97 (0.01)	0.58
Left lateral occipital	2.40 ± 0.09	2.35 ± 0.09	t = 3.52 (0.004)	0.56
Left supramarginal	2.73 ± 0.17	2.65 ± 0.18	t = 3.12 (0.008)	0.47
Left superior frontal	2.88 ± 0.15	2.82 ± 0.16	t = 3.19 (0.007)	0.40
Right superior frontal	2.80 ± 0.13	2.74 ± 0.15	t = 2.79 (0.015)	0.46
Right inferior parietal	2.76 ± 0.13	2.69 ± 0.15	t = 3.74 (0.002)	0.54
Right bank of sts	2.85 ± 0.18	2.78 ± 0.16	t = 3.47 (0.004)	0.39
Right fusiform	2.91 ± 0.12	2.79 ± 0.13	t = 5.01 (<0.0001)	1.00
Right insula	3.27 ± 0.18	3.14 ± 0.15	t = 5.37 (<0.0001)	0.72
Right precuneus	2.59 ± 0.14	2.48 ± 0.18	t = 3.93 (<0.002)	0.79

Only results with *P* ≤ 0.01 were considered.
[†]Mean ± SD
[‡]Effect size: 0.20 (small); 0.20–0.50 (medium); ≤0.50 (large)
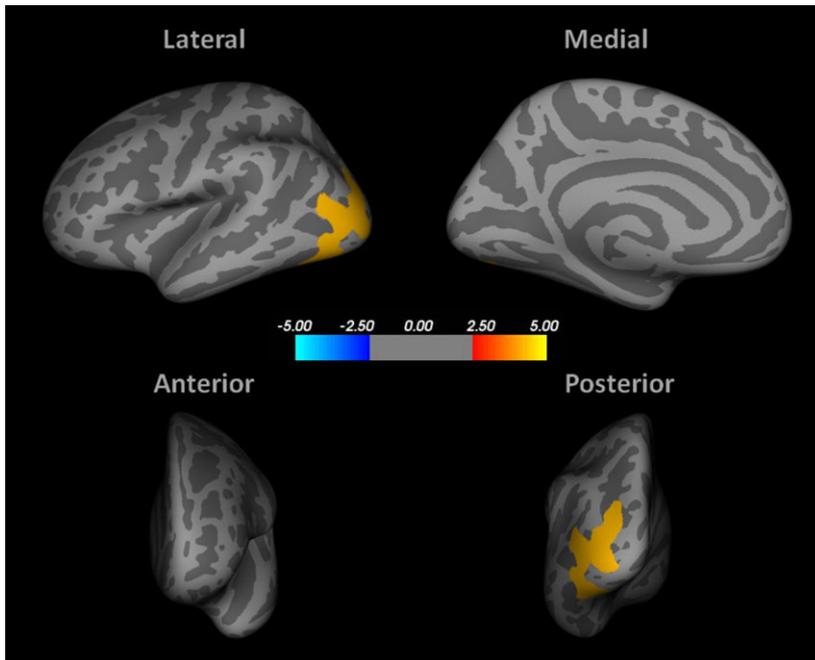sts = superior temporal sulcus

Figure 1 The correlation map analysis between the rate of changes in CTh of the left hemisphere and the rate of changes in the free testosterone index in female-to-male transsexuals. A positive correlation is seen in the lateral occipital, inferior and superior parietal and fusiform areas. The color bar represents statistically significant ($P < 0.05$) positive (yellow) correlations.

MtFs after the administration of estrogens and anti-androgens (Table 1). Serum testosterone decreased 96% and free testosterone index 96% while estradiol increased 79%. However, we did not find any significant correlation between changes in CTh and changes in hormonal levels. Regression analyses did not show any significant effect either.

Discussion

The current study describes for the first time the effects of cross-sex hormonal treatment on the brain gray matter of transsexuals. There are two main findings. First, FtMs showed thicker cortex regionally after testosterone treatment. This increment correlated positively with increments in the free testosterone index and the serum testosterone. Second, MtFs, after estrogens and antiandrogens treatment, showed a general thinner cortex as well as volumetric reductions in subcortical gray matter structures. Furthermore, an enlargement of the ventricular system was also observed.

Female-to-Male Transsexuals

Testosterone treatment is associated with increases in CTh that are bilateral in the postcentral gyrus, unilaterally left-sided in the inferior parietal, lingual, pericalcarine, and supramarginal regions, and unilaterally right sided in the cuneus and rostral middle frontal areas. These regions are known to be sexually dimorphic, females having a thicker cortex than males [6,36,37]. Moreover, these sex differences seem to be modulated from adolescence to adult life by testosterone and the AR, producing a different reduction in specific regions according to sex [37–39].

Some of these regions in which we have observed increases in CTh (cuneus, pericalcarine, lingual, and inferior parietal) are primary and associative visual cortices and are involved in visuoperceptual and visuospatial functions [40]. It is interesting to note that males are generally better than females in visuospatial functions while females are generally better than males in visuoperceptive ones [41]. Moreover, we observed increases in the postcentral region involving the somatosensorial cortex, which is associated with body perception [42]. The left supramarginal gyrus is involved in complex linguistic functions [43].

In a previous study, we observed that the CTh of FtMs before testosterone treatment was similar to that of control females and showed a greater CTh than males in the parietal and temporal cortices, suggesting that in FtMs the CTh, although not masculinized, does have phenotypic peculiarities [6].

As far as we know, with the exception of some specific regions in the white and gray matter [3,6], FtMs under hormonal treatment are anatomically female with supraphysiological pharmacologically-induced levels of testosterone. Although testoster-

one treatment in FtMs seems to produce few adverse effects [44], any attempt to explain the increase of CTh observed in FtMs after hormone treatment needs to underscore their male induced levels of testosterone. The heavy and continuous exposure of the FtMs' brains to testosterone might exceed the usual metabolic mechanisms of androgens and their metabolites in the brain.

Testosterone and its metabolites affect cell functions in the brain by complex and multiple routes. They use genomic and non-genomic pathways that are probably interlinked; the human brain has AR [7] and ER [14,45] receptors. Moreover, the mammalian brain, including humans', possesses most of the enzymes involved in the biosynthesis and metabolism of sex steroids and glucocorticoids [46].

We do not know if the increases in the CTh are promoted through the AR, the ER or both. Testosterone, and its reduced metabolite dihydrotestosterone, can act directly via the AR. However, other reduced metabolites (3α diol and 3β diol) bind to the ER. Moreover, testosterone is converted to estradiol in the brain via the p450 enzyme aromatase and, ultimately, act on the ER. Aromatase and reductase activities have been detected in the human brain [46–48].

In addition to the above summarized mechanisms that could explain the CTh increments observed in FtMs under androgenization treatment, it has been suggested that androgens exert an anticatabolic effect by interfering with glucocorticoids receptor expression [49], thus inducing a positive nitrogen balance. Glucocorticoids receptors have been identified in the human brain [50].

CTh reflects cellular characteristics of the neuropil such as number and size of cortical cells, including their packing density and also the degree of myelinization [51,52]. In physiological conditions a female's gray matter is sensitive to hormonal changes during the menstrual cycle [53] and pregnancy [54]. In FtMs androgenization increases total brain and hypothalamus volumes [17]. It could be that the supraphysiological levels of testosterone experienced by FtMs might enhance anabolic effects in cortical brain cells (size, myelination, packaging, etc) that were previously fine-tuned by the normal female levels of androgens.

It should be noted that testosterone has androgenic (virilizing) and anabolic effects that cannot be teased apart. FtMs are convinced that they are men before testosterone treatment and they have a feminine pattern of CTh with some peculiarities [6]. The main goal of testosterone treatment is to induce body changes (virilization in skin, a male pattern of hair and fat distribution, amenorrhea, and deepened voice) in order to obtain a masculine phenotype [20]. In addition, clitoral growth, increases of the body mass index, muscle mass and strength, and laryngeal prominence are observed [55,56]. All these body changes contribute to the psychic wellbeing of FtMs [20,57] and are due to both the virilizing and anabolic effects of testosterone. We believe that to account for the CTh increases observed in FtMs it is necessary to consider the anabolic effects of androgens. Testosterone and its androgenic metabolites stimulate protein synthesis and protein catabolism [58]. Indeed, in a previous report we have shown that FtMs androgenization induced an increase in FA values [59]. These increments in FA might reflect greater richness in axonal microtubules and macromolecules. Anabolic agents are clinically used to treat cachexia in chronic disease states and to control loss of muscle mass in the elderly. In sport, androgens induce performance enhancement in women [49,58]. Moreover, anabolic agents are also used by body builders. Thus, the CTh increments we observed in FtMs might be extended to both women and men that are chronically treated with anabolic agents.

Another finding of the study is that the increases in the levels of testosterone after treatment correlate with the increments in CTh in the left occipito-temporo-parietal region. This might suggest that these areas may be especially rich in ARs and ERs in humans. Moreover, these regions overlap with some regions in which we observed an increment of CTh. All these observations reflect that the higher the level of testosterone, the thicker the cerebral cortex in FtMs.

One relevant question is if the gray matter increase can be associated to behavioral effects. It has been reported that hormonal therapy in FtMs improves mental rotation ability [60] and the performance of some visual memory tasks [61] although an absence of effects has also been reported [62,63]. With respect to other types of behaviors, testosterone treatment in FtMs is associated to better mood, less anxiety and social distress [57] and increased sexual desire [64].

To the best of our knowledge, ours is the first study to demonstrate increases in CTh after testosterone treatment. However, there is growing evidence for the increase of gray matter after cognitive training in adults [65]. A substantial gray matter volume expansion has been reported in frontal and parietal brain areas after only two

practice sessions in a complex whole-body balancing task [66]. We do not know the anatomical and functional significance of the gray matter increments observed in MRI studies. However, it was suggested [67] that they probably reflect simple changes in neurons or glial cell size, the genesis of these cells, or synaptogenesis. On the other hand, it has also been suggested that MRI CTh reflects the number and size of cortical cells or their packing and degree of myelination [52]. Whether testosterone acts on this constellation of possibilities will need further studies.

Male-to Female Transsexuals

The second main finding of our study is that MtFs, after estrogens and anti-androgens treatment, showed a general reduction of gray matter in cortical and subcortical structures, accompanied by an enlargement of the ventricular system.

Reductions of gray matter were seen in both CTh and subcortical gray matter. We observed volumetric reductions in the right hemisphere, specifically in the pallidum and thalamus. In accordance with our study, in a sample of 8 MtFs, Hulshoff Pol et al. [17] also found a decrease of total brain volume and hypothalamus volume after hormonal treatment. Their sample also showed lower gray matter values posttreatment but the observation did not achieve statistical significance, probably because of the small sample size.

In MtFs we observed a global thinning of the whole cortex that was highly significant in the following regions: superior frontal (bilateral), bank of the superior temporal sulcus, rostral and isthmus cingulate, insula, fusiform, inferior parietal, supramarginal, lateral occipital and precuneus. Moreover, the right thalamus and the right pallidum showed a volumetric reduction. But, how can we explain the reduction of CTh and volumetric values of subcortical structures after estrogens and anti-androgens treatment? The association of estrogens and anti-androgens in the treatment of MtFs precludes a simple explanation of the results we found, but there are cues in the literature that could help their interpretation.

First, the effects of estrogens on gray matter volume as detected by MRI studies have been investigated in young and post-menopausal women and results coincide in that estrogens are associated with gray matter decreases. In pubertal girls, a higher estradiol level was associated with decreased density within prefrontal, parietal and middle temporal areas [68]. Gray matter volume increases and decreases in subcortical structures have been associated to pre [69] and postmenstrual phases of the cycle [53]. Furthermore, a significant gray matter volume peak was also reported at the time of ovulation [70]. Moreover, the brain decreases in size during pregnancy and increases after delivery [54]. All these reports show that the brain and sex steroids are tuned in physiological states. However, the picture is quite different in postmenopausal women under estrogen replacement therapy. Women under hormone therapy had significantly less gray matter than controls. In addition, estrogen therapy was associated to smaller regional volumes in frontal, temporal and limbic regions as well as the hippocampus [71,72]. Estrogens have been associated with greater brain atrophy among women 65 years and older [71].

Second, in FtMs we suggested that the increases in CTh we observed after testosterone treatment could be due to the anabolic and anticatabolic effects of testosterone and its metabolites including estradiol. If the anabolic effects of testosterone are responsible for the CTh increments observed in FtMs, the suppression of testosterone in MtFs via antiandrogens might also explain in part the observed decreases since the neural tissue is deprived of anabolic agents.

Third, the possible adverse effects of estradiol on the neural tissue have been described in animal models of adult and developing rat brain. In the arcuate nucleus, a sexually dimorphic structure [73], estradiol has been shown to be selectively cytotoxic to β-endorphin neurons. The mechanism that underlies this neurotoxic effect involves the aromatic hydroxylation of estradiol to catechol estrogens and a subsequent oxidation to o-semiquinone free radicals [74]. The developing human hypothalamus and cerebral cortex can convert estradiol to catechol estrogens [75].

Moreover, there is evidence that estradiol promotes neuronal death in the developing brain. With respect to effects of estradiol on the developing brain, it should be underscored that sexual dimorphism in the brain is expressed in two morphological patterns, one in which males show greater morphological measurements (Males > Females) and the opposite (Females > Males) [76]. In structures that present the F > M morphological pattern, which is the case of CTh in humans, newborn male gonadectomy produces an increase in the male morphological measures in the lateral and medial anterior regions of the bed nucleus of the stria terminalis, the parastrial nucleus, the anteroventral periventricular nucleus and the arcuate nucleus [77]. This suggests that testoster-

one or its aromatized metabolites (estradiol) exert "inhibitory" influences on specific brain regions. This "inhibitory" mechanism has been described in the developing hypothalamus for the anteroventral periventricular nucleus, with estradiol inducing neuronal cell death that is mediated by the ER [78]. Whether this inhibitory mechanism is also expressed in adulthood under supra physiological doses of estrogens has not been proven as yet.

After hormonal treatment MtFs also showed an enlargement of the ventricular system which can be explained as a consequence of the general reduction of the gray matter since we did not observe any change in global white matter. Increases of the ventricular system associated to gray matter decreases are the normal pattern associated to aging [79] suggesting an *ex vaquo* mechanism. Both reductions of gray matter due to cell death or shrinkage can be responsible of ventricular enlargements. It has been reported that antipsychotic drugs reduce the volume of gray matter and increase the ventricular volume although the mechanism is unknown [80].

In MtFs we did not find correlations between changes in hormonal levels and changes in CTh. For testosterone the lack of correlation was due to the absence of significant variability in the percentage of hormonal decreases. Changes in estradiol did not correlate either. This was due to the fact that some subjects showed increased estradiol levels while others showed decreases after treatment. Significant interindividual variations have been reported after administration of estradiol [81].

General Comments

An important question is the general scope for the interpretation of our results. The brains of FtMs and MtFs already have a specific phenotype before the cross-hormone treatment [3,4,6]) and their gender identity feelings are well established before the treatment is instituted. The hormone treatments are prescribed to masculinize (FtMs) or feminize (MtFs) their bodies. So the treatment effects we observed in their CTh and the volume of subcortical structures ought not to be associated to a strengthening of their awareness of their gender. This picture is supported by other behavioral functions that have been studied. For instance, in mental rotation tests men outperform women [82]. These tests have been used to study the possible masculinization or feminization of spatial abilities in pre- and posttreated transsexuals. Curiously, the cross-sex hormonal treatment does not affect the posttreatment performance [62,83,84] even after a chronic hormonal treatment [63]. Thus, it seems that in transsexuals some sexually dimorphic behavioral functions (i.e., gender identity, spatial abilities) that are already established pretreatment are not affected by cross-sex hormonal treatment.

At least three questions emerge from our results. Are the changes in CTh we observed reversible? Are there clinical correlates for these changes? How will long-term treatment affect cerebral structures? From our design we cannot provide an answer. However, we can introduce some speculations that might help future research.

First, if the CTh increases seen in FtMs are mainly due to the anabolic effects of testosterone one would suspect that withdrawal from the treatment might return the CTh to pretreatment values. In MtFs, CTh was decreased after anti-androgens and estrogens treatment. In this case, we provide two combined hypothetical explanations: (a) a CTh decrease produced by a fall in the anabolic effects of testosterone due to the administration of antiandrogens and, (b) the probable adverse effects of estrogens on gray matter by means of catechol estrogens. The weight of these possible mechanisms would determine the reversibility of the CTh thinning. Recently, using resting-state fMRI it was found that in one FtM transsexual that his functional connectivity profile was comparable to female control subjects [85]. Future research with larger samples should clarify if the CTh and volume changes we see in FtMs and MtFs after cross-sex hormone treatment also affect functional cortical connectivity.

Second, from the clinical perspective it has been stated that cross-sex hormone treatment of transsexuals seems acceptably safe over the short and medium term, but solid clinical data are lacking [20]. Most of the existing behavioral studies of cross-hormone therapy focus on cognitive functions that are sexually dimorphic (such as spatial abilities or verbal fluency) and on psychopathology (changes in mood, anxiety, sexual interest). Estrogen treatment associated with sex change in MtFs has little or no influence on sex-typed aspects of cognition or memory [84]. Moreover, MtFs receiving hormonal therapy showed less psychopathology on MMPI scales than untreated patients [86,87] but there is a report that observes increases in depressive mood [88]. Both MtFs and FtMs under cross-hormone treatment show less social distress, anxiety and depression those patients without treatment [57]. A positive effect

of testosterone on mood and on sexual function has also been described [89]. We have seen robust effects of increases (FtMs) and decreases (MtFs) in CTh after the cross-sex treatments; our findings might help to choose new and more sensitive neuropsychological explorations that would be useful to confirm the absence of behavioral effects of cross-sex hormone treatment.

With respect to the third question, there is a study about the long-term cross-sex hormone use; FtM did not experience important side effects such as cardiovascular events while a quarter of MtFs had osteoporosis and 6% tromboembolic and cardiovascular problems [90]. Our designs offer results on CTh for short and medium terms. The hormonal treatment usually lasts the whole life. At this time we can not speculate on the course that CTh increases (FtMs) and decreases (MtFs) will follow with chronic treatment.

Finally, one laboratory has approached the brain of MtFs through histological *post mortem* material. This group showed sex differences in the volume and number of neurons in the central part of the bed nucleus of the stria terminalis [BSTc; 91,92] and the interstitial nucleus of the anterior hypothalamus number 3 [INAH3;93], with males having greater morphological measurements than females. Moreover, they have also communicated volumetric and neuronal number decreases in the BSTc and INAH3 in cross-sex hormone treated MtFs [91–93]. They hypothesized that their findings were independent of the levels of circulating sex hormones [91,92] and that the feminization of these nuclei occurred early in development. Our results show that the cross-sex hormone treatment is related to morphological decrements in total gray matter, cortical gray matter volume and the volume of the right thalamus and pallidum. In addition, we also found a decrease in the cortical thickness. The studies from Swaab' laboratory are cross-sectional designs on *post mortem* brain specimens and the effects of the cross-sex hormone treatment cannot be discarded from their MtFs subjects. However, our design is an *in vivo* pre-post design that does allow us to see the effect of the hormonal treatments; we found an effect of the cross-sex hormone treatment in the CTh of both MtFs and FtMs. Moreover, in MtFs the CTh decreases are associated to a global increase in the ventricular system.

Strengths and Limitations

This is the first study to show the effects of high doses of cross-sex hormones on the CTh in adult biologically male and female humans. The cortical mantle represents eighty per cent of the total gray matter of the brain. Our study has some limitations. First, it could be argued that the absence of untreated control groups to evaluate possible changes in CTh due to the influence of maturation CTh is a limitation. However, the time of evolution seems to be unrelated to changes in CTh since we observed clearly opposite directions over time in the CTh of FtMs and MtFs groups after cross-sex hormonal treatments. Moreover, the effects observed in both groups exceed those reported in the literature on the influence of time on CTh. We have seen that after testosterone treatment FtMs show a thicker cortex. From adolescence on there is a progressive decrease of CTh in almost all cerebral regions [33]. A great extent of the cortical mantle shows thinning rates of at least 0.01 mm/decade [33]. Thickening of the cortex has also been described with increasing age in the anterior cingulate and medial orbitofrontal subcallosal cortex [33], but we have not observed CTh increases in these areas. In consequence, the effect of time *per se* between pre- and posttreated FtMs does not seem to explain the changes in CTh because in the regions in which we observed thickening, the expected age effect would have been thinning.

In relation to the effect of time in MtFs, the comparison between the cerebral structures pre- and post hormonal treatment in this group showed a reduction in the global volume of the gray matter. The percentage of total gray matter volume reduction was 3% in six to thirty months and is near the expected reduction of CTh in a decade in adult subjects [33]. The reduction in normal older people is 0.5% by year [94]. Thus, the results we report seems to be due to the hormonal treatment, because they are stronger than those expected from the aging effects on CTh [95].

Finally, another limitation is the number of comparisons that we have carried out in the study of the cortical areas using the Desikan-Killiany atlas [30]. We did not apply the Bonferroni correction because of the exploratory nature of our study, as well as the small sample size, and the interdependence of the analyzed cerebral variables [96,97].

Conclusion

Testosterone treatment in FtMs is associated to an increment of the CTh in specific posterior regions of the cortex. Moreover, this thickening is corre-

lated to changes in testosterone levels in part of these regions of the cortex.

In MtFs, estrogens and antiandrogens are associated to a decrease in the CTh and subcortical structures that consequently induces an enlargement of the ventricular system. Our study helps to identify the cortical regions that are most sensitive to the effects of sexual hormones in FtM and MtF transsexuals. Moreover, it might be extended to those receiving anabolic drugs. Future studies might evaluate the clinical relevance of these morphological changes in the cerebral cortex after long-term cross-sex hormone long administration.

Corresponding Author: Antonio Guillamon, MD, PhD, Departamento de Psicobiologia, UNED, c/ Juan del Rosal, 10, 28040 Madrid, Spain. Tel: (34) 91-398-6272; Fax: (34) 91-398-6287; E-mail: aguillamon @psi.uned.es

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Statement of Authorship

Category 1

(a) Conception and Design
Carme Junque; Esther Gómez-Gil; Antonio Guillamon

(b) Acquisition of Data
Leire Zubiaurre-Elorza

(c) Analysis and Interpretation of Data
Leire Zubiaurre-Elorza; Carme Junque; Esther Gómez-Gil; Antonio Guillamon

Category 2

(a) Drafting the Article
Leire Zubiaurre-Elorza; Carme Junque; Esther Gómez-Gil; Antonio Guillamon

(b) Revising it for Intellectual Content
Leire Zubiaurre-Elorza; Carme Junque; Esther Gómez-Gil; Antonio Guillamon

Category 3

(a) Final Approval of the Completed Article
Leire Zubiaurre-Elorza; Carme Junque; Esther Gómez-Gil; Antonio Guillamon

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