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Subcortical gray matter changes in transgender subjects after long-term cross-sex hormone administration

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ABSTRACT

Sex-steroid hormones are primarily involved in sexual differentiation and development and are thought to underlie processes related to cognition and emotion. However, divergent results have been reported concerning the effects of hormone administration on brain structure including side effects like brain atrophy and dementia. Cross-sex hormone therapy in transgender subjects offers a unique model for studying the effects of sex hormones on the living human brain. In this study, 25 Female-to-Male (FtM) and 14 Male-to-Female (MtF) subjects underwent MRI examinations at baseline and after a period of at least 4-months of continuous cross-sex hormone administration. While MtFs received estradiol and anti-androgens, FtM subjects underwent high-dose testosterone treatment. The longitudinal processing stream of the FreeSurfer software suite was used for the automated assessment and delineation of brain volumes to assess the structural changes over the treatment period of cross-sex hormone administration. Most prominent results were found for MtFs receiving estradiol and anti-androgens in the form of significant decreases in the hippocampal region. Further analysis revealed that these decreases were reflected by increases in the ventricles. Additionally, changes in progesterone levels correlated with changes in gray matter structures in MtF subjects. In line with prior studies, our results indicate hormonal influences on subcortical structures related to memory and emotional processing. Additionally, this study adds valuable knowledge that progesterone may play an important role in this process.

1. Introduction

Sex-steroid hormones are involved in sexual differentiation, development and behaviour (Zubiaurre-Elorza et al., 2014) and play a pivotal role in the development and function of the central nervous system (Paus et al., 2010; Peper et al., 2009). They exert varied effects on the brain and the body and are thought to alter several processes related to cognition and emotion (Höfer et al., 2013; Toffoletto et al., 2014). For example, higher levels of progesterone and estradiol during pregnancy have been associated with a worsening of mood and an impairment of memory performance (Buckwalter et al., 1999; van Wingen et al., 2008). Studies also revealed an impact on emotional processing, as indicated by alterations in amygdala activation due to changing hormonal levels during the menstrual cycle (Derntl et al., 2008). These results were also substantiated by the fact that cognitive changes have been associated with hormonal contraceptive use. Specifically, performance changes on verbal memory, verbal fluency and on the mental rotation task in woman using oral contraceptives have been observed (Griksiene and Ruksenas, 2011).

Gonadal hormones either act as neuroactive steroids by modulating ligand-gated ion channels and G-protein coupled receptors or by binding to nuclear androgen (Beyenburg et al., 2000; Finley and Kritzer, 1999; Puy et al., 1995) and estrogen receptors (González et al., 2007; Montague et al., 2008; Osterlund et al., 2000a), directly influencing gene expression (Brinton et al., 2008; Rupprecht and Holsboer, 1999). These receptors have been detected in gray matter (GM) cortical areas as well as in subcortical

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structures (Fernández-Guasti et al., 2000; Finley and Kritzer, 1999; Kruijver et al., 2002; Osterlund et al., 2000b; Puy et al., 1995).

Animal studies already indicated influences of sex-steroid exposure on brain morphology. In this regard, hippocampal synaptic plasticity and neurogenesis in rodents after testosterone and estrogen administration has been observed (Galea et al., 2006; Gould et al., 1990; MacLusky et al., 2006).

In addition, sex hormones influence neural development during puberty and in the adult human brain. For example, increasing levels of circulating testosterone during puberty in boys indicate a contribution to sex differences in the amygdala and hippocampus region during adolescence (Neufang et al., 2009). It was further shown that circulating sex hormones were related to GM structures in several areas of the brain, indicating an influence of steroid hormones on brain morphology in the human brain (Witte et al., 2010).

Treatment studies involving humans are scarce, due to ethical and methodological reasons. However, a unique model to study the influence of long-term high-dose sex-steroid hormone treatment onto the living human brain can be achieved by the investigation of Female-to-Male (FtM) and Male-to-Female (MtF) transgender people. These subjects are characterized by strong and persistent cross-gender identification, experience an incongruency between their biological sex and their gender identity, finally seeking hormonal treatment and in some cases sex reassignment surgery (Bao and Swaab, 2011).

First evidence for a putative influence of cross-sex hormone treatment on brain structures in transgender subjects was observed in post-mortem studies. It was shown that the bed nucleus of the stria terminalis of the hypothalamus was of female size in MtFs and of male size in one observed FtM subject, which may have been attributable to cross-sex hormone administration (Kruijver et al., 2000; Zhou et al., 1995). So far, only two studies have investigated the influence of long-term high-dose cross-sex hormone treatment on gray matter brain morphology in FtM and MtF transgender individuals in vivo. Pol et al. showed that testosterone in FtM subjects increased total brain volume and the hypothalamus, whereby estrogens and anti-androgens in MtF subjects led to decreases in brain volume and to an increase in the ventricles. Authors concluded that testosterone led to masculinization, whereby estradiol and anti-androgens to feminization of the brain (Pol et al., 2006). However, sample size was rather small, with only 8 MtF and 6 FtM transgender participants and a limited number of brain structures have been evaluated. Detailed results were delivered by a more recent study, where it was shown that testosterone therapy increases cortical thickness in FtM subjects (Zubiaurre-Elorza et al., 2014). The thickening in cortical regions was associated with changes in testosterone levels. On the other hand, estrogens and anti-androgen therapy in MtFs was associated with a decrease in cortical thickness. But also subcortical structures were affected in the form of GM increases in the thalamus after testosterone administration and decreases in the thalamus as well as in the pallidum due to estradiol and anti-androgen treatment. Interestingly, also an enlargement of the ventricles was observed in the MtF cohort.

Taken together, studies on the influence of sex hormones in transgender individuals are scarce and limited by small sample sizes. Moreover, only uncorrected results have been reported so far in the literature. Here, we used the longitudinal processing stream implemented in FreeSurfer to increase statistical power by reducing the confounding effect of between-subject variability. Based on prior observations, we expected GM decreases due to estradiol and anti-androgen treatment and testosterone induced increases in gray matter structures, while for ventricular structures the opposite effect is expected.

2. Methods

2.1. Subjects

29 FtM and 21 MtF transgender participants underwent MRI assessment after the screening phase. However, 4 FtM (mean age ± SD = 28.5 ± 7.2) and 7 MtF (32.8 ± 10.0) subjects had to be excluded due to early study termination after the first measurement or movement artefacts during scanning. Hence, structural brain changes of 25 FtM (27.1 ± 6.0) and 14 MtF (26.9 ± 6.1) transgender participants were finally analysed in this longitudinal study. The second measurement was carried out at least after 4 months of continuous cross-sex hormone administration while FtM subjects had slightly larger intervals between the scans (days ± SD = 180 ± 130) than the MtF population (169 ± 38). Furthermore, 14 female (mean age ± SD = 24.9 ± 4.9) and 12 male subjects (28.0 ± 5.9) were included as control participants and were also measured at two time points (days ± SD = FC: 95 ± 34.2; MC: 85 ± 40.5).

Structural data from a subsample of subjects have been published in two previous cross-sectional studies (Hahn et al., 2015; Kranz et al., 2014). Participants were recruited from the Transgender outpatient unit of the Department of Obstetrics and Gynecology, Medical University of Vienna. All subjects were naïve to steroid hormone treatment and transgender participants reported onset of gender dysphoria before or at puberty. Subjects underwent standard medical examinations including electrocardiogram, routine laboratory tests and the Structural Clinical Interview for DSM-IV Disorders. Exclusion criteria included (1) intake of psychotropic medication and hormones (prior to baseline measurement), (2) past or current substance abuse, (3) pregnancy, (4) history of psychiatric, neurological, or medical disorders and (5) MRI contraindications.

The diagnosis of gender identity disorder was assessed according to the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) by an experienced psychiatrist at the screening visit. It is stated as a strong and persistent (>6 months) cross-gender identification and discomfort with the current sex, causing clinically significant distress or impairment in social, occupational or other areas of functioning. All participants provided written informed consent after detailed explanation of the study protocol. This study was approved by the Ethics Committee of the Medical University of Vienna and procedures were performed according to the Declaration of Helsinki.

2.2. Study design and treatment protocol

The study was designed as a longitudinal monocentric study. All transgender participants underwent a baseline MRI scan before start of hormone treatment and were measured again after 4 months of high-dose cross sex hormone administration. Control subjects were also measured at a second time point within 4 months but without receiving any hormonal treatment.

Hormone treatment followed protocols routinely implemented at the Department of Obstetrics and Gynecology, Unit for Gender Identity Disorder, at the Medical University of Vienna. While FtM subjects underwent testosterone treatment, MTFs received estradiol and anti-androgen medication. Specifically, FtM subjects received either 1000 mg testosterone undecanoate every 12 weeks (Nebido 250 mg/ml, 4 ml vial, intramuscular) or 50 mg testosterone daily (Testogel 50 mg/5 mg bag, transdermal). If menstruation still persisted, additionally either 10–15 mg lynestrenol (Orgametril 5 mg, oral) or in some cases 75 μg desogestrel (Cerazette 75 μg, oral) daily were administered.

MtF participants were treated with daily 50 mg cyproterone acetate (Androcur 50 mg tablet, oral). Additionally, 4 mg/day

estradiol hemihydrate (Estrofem 2 mg, oral) was administered. Alternatively, some participants received estradiol hemihydrate 0.75 mg/day–1.5 mg/day (Estrogel-Gel 75 mg/1.25 g/day, transdermal). In some cases triptorelin acetate 4.12 mg/month (Decapeptyl 172 mg powder for suspension for injection subcutaneous or intramuscular) was administered. Due to extensive hair loss, some MtFs further received 2.5 mg of the 5-alpha reductase inhibitor finasteride (5 mg, oral; Ratiopharm) every second day.

2.3. Serum sampling

Blood samples were collected at the baseline and at the follow-up MRI scans. The analysis of plasma levels of progesterone, estradiol and testosterone was done by the Department of Laboratory Medicine, Medical University of Vienna, Austria (http://www.kimcl.at).

2.4. Data acquisition

Structural MRI was performed at the Magnetic Resonance Centre of Excellence at the Medical University of Vienna, Austria. Subjects underwent structural MRI at a 3 T whole-body scanner (Siemens Tim Trio, Erlangen, Germany) using a 32-channel head coil and a T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence (160 slices, 256×240 matrix, voxel size $1.1 \times 1 \times 1\ mm^3$, TE = 4.21 ms, TR = 2300 ms; TI = 900 ms; $\alpha = 9^\circ$; total acquisition time 7 min, 46 s).

2.5. Surface-based and volume based analysis with FreeSurfer

For the cortical and subcortical assessment, the standard pipeline of the FreeSurfer software suite (http://surfer.nmr.mgh.harvard.edu/, version 5.1.0) was used. In short, in the cortical based pipeline (Dale et al., 1999; Fischl et al., 1999), every volume of a subject is registered with Talairach atlas (Talairach and Tournoux, 1988) using an affine registration. After bias field correction, skull stripping is performed using a deformable template model (Segonne et al., 2004). During that step, hemispheres are separated and cerebellum and brain stem are removed. Then white matter is segmented. White and pial surfaces are estimated and the distance between them is used to calculate the thickness of each location at the cortex (Fischl and Dale, 2000).

The subcortical stream shares several steps (Talairach registration, bias field correction) with the cortical approach, but different algorithms are used to label subcortical tissue classes (fully described in Fischl et al., 2004, 2002). After the cortical and subcortical automated stream, all volumes were visually inspected to maintain high quality segmentations.

As subjects were measured at two time points, images were then processed with the longitudinal stream (Reuter et al., 2012). Here, an unbiased within-subject template space and image (Reuter and Fischl, 2011) is created by using robust, inverse consistent registration (Reuter et al., 2010). Talairach transforms, atlas registration as well as spherical surface maps and parcellations are then initialized with common information from the within-subject template. These steps were shown to significantly increase reliability and statistical power (Reuter et al., 2012).

2.6. Statistical analysis

Linear mixed models were used to assess the influence of high-dose cross sex hormonal treatment on subcortical gray matter structures with group (FtM, MtF) as between subjects factor, time (baseline, treatment) and ROI (14 subcortical structures calculated within FreeSurfer) as repeated factors and subject as random factor. The same analysis has been carried out for the ventricular structures, with the repeated factor ROI (4 ventricular structures). The significance level was set at 5% in all analyses. SPSS version 22.0 for Windows (SPSS Inc., Chicago, Illinois; www.spss.com) was used for statistical analysis. As prior studies indicated decreases in gray matter structures in MtFs and increases in FtMs (Pol et al., 2006; Zubiaurre-Elorza et al., 2014), one-tailed tests were applied. To determine linear relationships between the changes of serum hormonal levels and changes in gray matter of each ROI, correlational analysis has been conducted in the regions, which showed significant GM changes over the two time points. To assess changes in the cortical regions, a vertex-wise surface-based analysis has been performed by using paired *t*-tests for each transgender group between the two time points. This was followed by a cluster-wise correction for multiple comparisons as proposed by Hagler et al. (2006).

3. Results

3.1. Study sample and scanning intervals

The transgender subjects (4 FtM, 7 MtF) excluded due to early study termination after the first measurement or movement artefacts during scanning did not differ significantly in terms of age compared to the final study sample (*t*-test; $p > 0.05$). The larger inter-scan variability of the FtM cohort compared to the MtF group was mainly driven by one of the 25 FtM participants. The re-analysis of the data excluding this participant did not change the main findings. As control subjects did not receive hormonal treatment and no structural changes were expected, scanning intervals were chosen more liberal compared to the transgender cohort. Intervals were shorter compared to the transgender group but did not differ significantly between female and male controls ($p > 0.05$).

3.2. Hormonal level changes

The blood hormonal levels of estradiol, testosterone and progesterone changed significantly (paired *t*-tests, all $p < 0.05$) after the treatment period in both transgender groups. In MtFs an increase in estradiol (TP1: 29.77 ± 14.42 pg/ml, TP2: 133.54 ± 121.33 pg/ml) was observed, testosterone (TP1: 5.48 ± 2.05 ng/ml, TP2: 0.97 ± 1.84 ng/ml) and progesterone (TP1: 0.76 ± 0.28 ng/ml, TP2: 0.53 ± 0.19 ng/ml) levels decreased. In FtM participants decreases in estradiol (TP1: 117.58 ± 70.84 pg/ml, TP2: 69.46 ± 61.94 pg/ml) and progesterone (TP1: 5.55 ± 6.75 ng/ml, TP2: 1.85 ± 2.94 ng/ml) were observed, while testosterone (TP1: 0.35 ± 0.15 ng/ml, TP2: 4.51 ± 2.50 ng/ml) levels increased markedly (Fig. 1). No significant changes in hormonal levels were found in both control cohorts (paired *t*-tests, all $p > 0.1$). For the FC group the following levels were observed: Estradiol (TP1: 130.31 ± 133.09 pg/ml, TP2: 144.62 ± 91.45 pg/ml), testosterone (TP1: 0.36 ± 0.09 ng/ml, TP2: 0.30 ± 0.16 ng/ml) and progesterone (TP1: 3.00 ± 5.25 ng/ml, TP2: 3.66 ± 4.14 ng/ml). One participant from the FC group was not included in the analysis as no blood was drawn at the second measurements due to technical difficulties. The MC cohort showed the following results: Estradiol (TP1: 25.58 ± 10.93 pg/ml, TP2: 25.25 ± 10.19 pg/ml), progesterone (TP1: 0.91 ± 0.51 ng/ml, TP2: 0.96 ± 0.60 ng/ml) and testosterone (TP1: 6.12 ± 1.61 ng/ml, TP2: 5.82 ± 1.99 ng/ml).

3.3. Cortical analysis

The surface-based vertex-wise analysis in the transgender group did not yield significant results when corrected for multiple comparisons. However, global decreases of cortical thickness

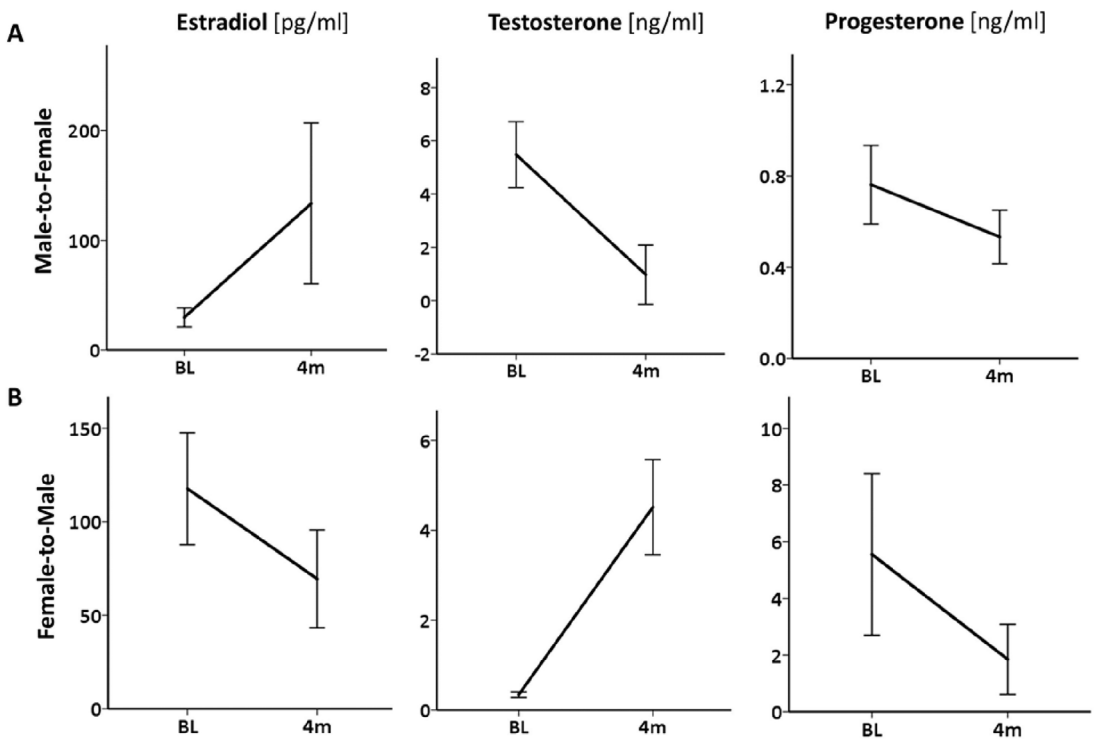

Fig. 1. Changes of blood serum hormonal levels after the 4-months treatment period (4m) compared to baseline (BL) in (A) MtF and (B) FtM subjects. Estradiol, testosterone and progesterone levels changed significantly after administration compared to baseline. In MtF subjects, estradiol levels increased, while testosterone and progesterone showed decreases. In FtM participants, estradiol and progesterone levels decreased, while testosterone increased. Lines represent mean with 95% confidence intervals.

in MtFs and increases as well as decreases in FtM subjects were observed across several cortical regions ($p < 0.05$, uncorrected).

3.4. Subcortical gray matter analysis

The subcortical analysis revealed a significant group x time interaction $F(1, 52.3) = 8.677$, $p = 0.005$ for transgender subjects. To assess the specific hormonal influences in each group, mixed models were then conducted separately for MtF and FtM subjects, followed by post-hoc comparisons between region and time point.

Analysis of MtF subjects showed a trend for time × ROI $F(13,38.4) = 1.802$; $p = 0.078$. However, time indicated a significant main effect $F(1,13.2) = 7.443$, $p = 0.017$. The post-hoc analysis revealed gray matter decreases in all subcortical structures, but not all areas reached significance. The post-hoc paired *t*-tests revealed significant results bilaterally in the hippocampus, amygdala and in the right caudate and putamen. After applying the Bonferroni correction for multiple comparisons, only the right hippocampus showed significant findings ($p = 0.028$) (Table 1, Fig. 2).

Analysing FtM participants, the mixed model analysis did neither reveal an interaction $F(13,74) = 1.079$; $p = 0.39$ nor a time effect $F(1,25.4) = 0.491$; $p = 0.49$ for treatment. However, exploratory paired *t*-tests for each region and time point revealed significant increases for the left thalamus and bilaterally for the pallidum. None of the tests in these structures survived correction for multiple comparisons (Table 1).

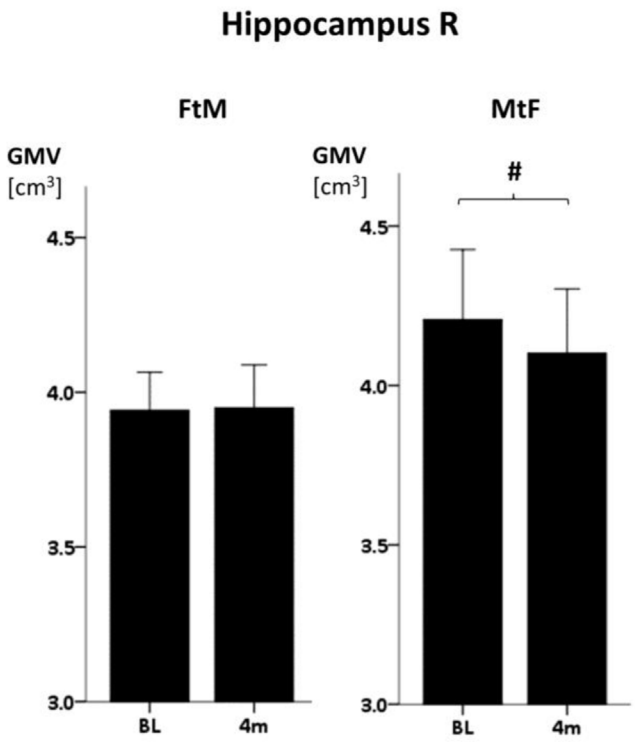

Fig. 2. Significant volume decreases in the right hippocampus in MtF subjects after the four months period (4m) of hormone administration compared to baseline (BL). No changes in FtM subjects were observed in this region. Bars represent mean with 95% confidence intervals. (#) Indicate significant results corrected for multiple comparisons.

3.5. Ventricular analysis

As prior literature indicated hormone induced changes in the ventricular system, we also analysed these structures for both transgender groups. Again, a linear mixed model was applied including the major ventricular structures (Left lateral ventricle, right lateral ventricle, third ventricle and fourth ventri-

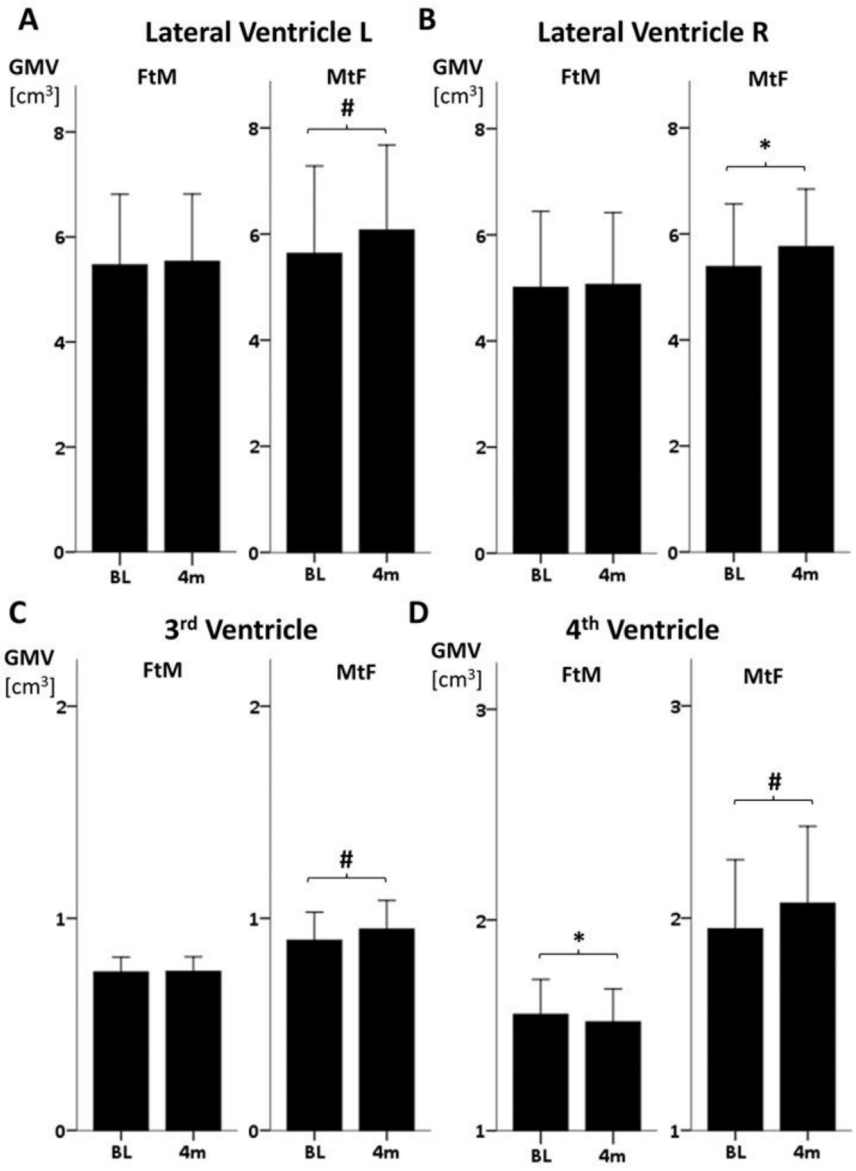

Fig. 3. Analysis of ventricular structures showed a trend for increasing ventricles in the MtF cohort after the treatment period (4m) in comparison to the baseline (BL) measurement, while ventricles in FtM subjects remained on the same level or showed slight decreases, as in the 4th ventricle. Bars represent mean with 95% confidence intervals. (#) Indicate significant results corrected for multiple comparisons, while (*) reports uncorrected *p*-values.

cle). Our analysis revealed a significant group × time interaction $F(1,31.2) = 8.626$, $p = 0.006$. Follow-up post-hoc group comparisons showed that this effect was mainly driven by an increase in MtF subjects. Significant results ($p < 0.05$) were found for ventricle increases in the 3rd ventricle (corr.), 4th ventricle (corr.) and bilaterally in the lateral-ventricles, left (corr.), right (uncorr.) while ventricles in FtM subjects only showed significant decreases in the 4th ventricle (uncorr.) (see Table 1, Fig. 3).

3.6. Correlational analyses between changes in serum hormone levels and changes in gray matter

As for 2 transgender subjects no hormonal status was assessed due to technical difficulties 1 MtF and 1 FtM had to be excluded from the correlation analysis. Correlations were then calculated for gray matter regions, which showed significant ($p < 0.05$) changes due to hormonal treatment in either group. In MtF subjects, correlations with testosterone changes ($r = 0.59$) were observed in the right amygdala but not with estrogen or in other regions. However, we observed interesting progesterone effects in the form of positive significant correlations in the right hippocampus ($r = 0.63$) and the right caudate ($r = 0.65$) (see Fig. 4). No significant correlations between changes in plasma levels and gray matter were observed in FtM subjects (Correlations reported, $p < 0.05$, uncorr.).

3.7. Subcortical gray matter and ventricular analysis in control subjects

To estimate effects not related to sex hormone treatment, gray matter and ventricular volume analysis were also performed in the two control cohorts. No significant effects were detected for FC or MC. The analysis of subcortical gray matter structures in FCs showed no significant interaction of time × ROI $F(13,31.1) = 0.681$; $p = 0.77$ and also the main effect $F(1,12.7) = 0.657$; $p = 0.433$ between the two time points was not significant. This also applied to the MC cohort. Although a time × ROI interaction $F(13,26.8) = 1.788$; $p = 0.09$ showed a distinct trend toward significance, no main effect for time $F(1,6.7) = 0.86$; $p = 0.386$ was

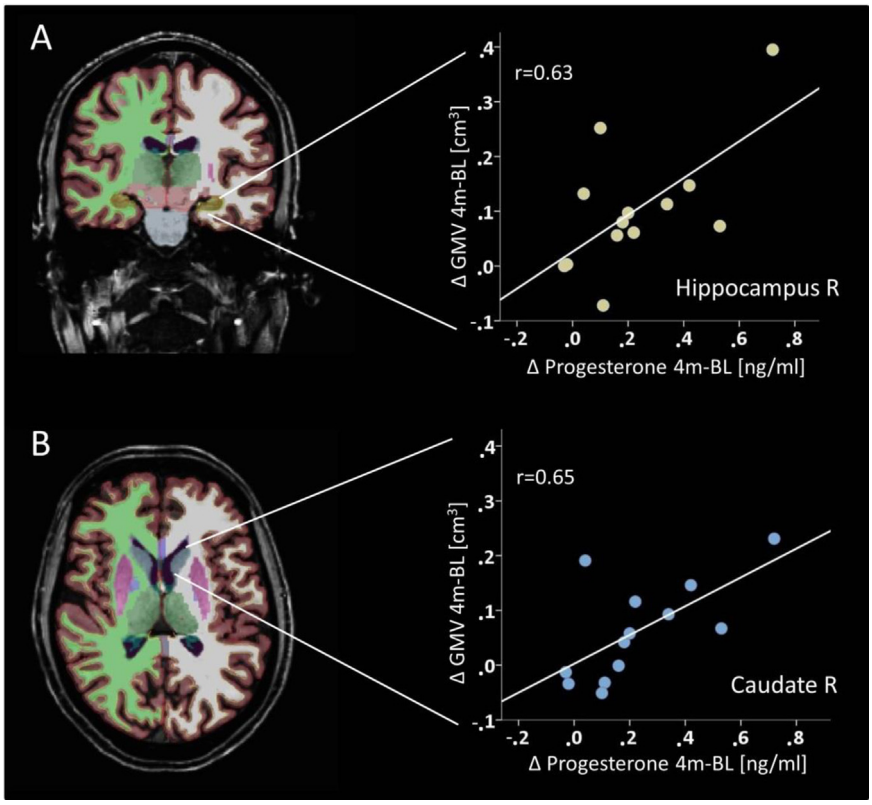

Fig. 4. Coronal and axial section of a single MtF subject with labelled subcortical regions automatically processed by the FreeSurfer software suite. Analysis revealed significant correlations between changes in progesterone and (A) changes in the right hippocampus and (B) changes in the right caudate nucleus.

found. The mixed model analysis of the ventricular structures in FCs delivered the following results for time × ROI interaction $F(3,34.0) = 1.247$; $p = 0.308$ without a main effect $F(1, 26.9) = 0.467$; $p = 0.5$, for MCs and also no significant interaction $F(3,14.7) = 0.504$; $p = 0.685$ or main effect $F(1,10.9) = 0.785$; $p = 0.395$ were found.

4. Discussion

The analysis of brain structures in MtF subjects, receiving estradiol and anti-androgen treatment for a period of at least 4 months, revealed GM decreases in the right hippocampus and increases in the ventricular system (corr.). Our results are generally in line with previous studies investigating transgender participants, where estradiol and anti-androgens mainly induced decreases, while testosterone led to increases in subcortical brain areas after cross-sex hormone administration (Pol et al., 2006; Zubiaurre-Elorza et al., 2014). Pol et al. for example concluded that in FtMs, the female brain size increased towards male proportions and vice versa for MtF subjects. Accordingly, decreases in total brain volume in MtF subjects (uncorr.) and a simultaneous increase in the third and lateral ventricles was observed in our current study. While our results in FtM subjects did not show general thicker cortices after testosterone treatment as proposed by Zubiaurre-Elorza et al. (2014), our investigations support the notion, that testosterone led to increases as well as decreases in cortical areas. However, we could corroborate findings of this study in the form of decreases in subcortical regions for MtFs. They showed reductions in subcortical regions such as the pallidum and thalamus, while our results were strongest in the hippocampus. While these two prior studies were limited by small sample sizes or reported only data not corrected for multiple testing, the present study presents evidence for changes in subcortical regions due to cross-sex hormone administration in MtF transgender participants.

The most prominent effect on subcortical structures in MtF subjects due to anti-androgen and estradiol treatment was found in the hippocampus, a region highly affected by processes related to neurogenesis and plasticity (Eriksson et al., 1998). In this regard, animal studies already indicated a substantial hormonal influence on hippocampal structure. It was shown that hippocampal synaptic plasticity and neurogenesis in the adult brain are dependent on testosterone and estrogens (Galea et al., 2006; MacLusky et al., 2006; Witte et al., 2010), including synaptic remodelling (Woolley and McEwen, 1992; Yankova et al., 2001), gliagenesis and neurogenesis (Gould et al., 2000). It has further been shown that androgen deprivation in rodents and primates led to decrease of spine synaptic density in the hippocampus. Interestingly, testosterone replacement again normalized the spine synaptic density levels (Leranth et al., 2003), indicating a strong modulation of this subcortical brain area due to hormonal exposure. Hence, it is no surprise that main structural changes have been observed in this brain region, as it is especially susceptible to hormonal action due to the large amount of steroid hormone receptors in this area. As MtF participants received estradiol and also anti-androgenic treatment, we assume that the decreased androgen levels are responsible for the observed structural decreases.

But also studies in humans, where circulating hormonal levels were observed, indicated influences on gray matter brain structures. Neufang and colleagues showed that testosterone levels during puberty affects regions like the amygdala and the hippocampus (Neufang et al., 2009), while (Peper et al., 2009) found that changes in gray matter also correlated with changes in hormonal levels. Another study could show that women under hormone therapy exhibited less gray matter compared to controls in the hippocampus (Resnick et al., 2009).

Evidence in rodents indicate that these effects are mediated by estradiol and progesterone receptors (Kato et al., 1994; Osterlund

Table 1
Changes in subcortical gray matter and ventricular brain volumes in MtF and FtM subjects. Presented t-values are one-sided, as in line with a priori hypotheses. Significant results (uncorrected) are marked with (*), while results corrected for multiple comparisons using the Bonferroni method are marked (#). All values are reported in cm^3.

Male-to-Female			
Subcortical structure	Baseline (mean ± sd)	Treatment (mean ± sd)	t-value
Hippocampus L	4.11 ± 0.32	4.06 ± 0.33	−2.27*
Hippocampus R	4.21 ± 0.38	4.10 ± 0.35	−3.47#
Amygdala L	1.71 ± 0.23	1.65 ± 0.21	−2.29*
Amygdala R	1.66 ± 0.16	1.61 ± 0.15	−2.64*
Caudate L	3.94 ± 0.39	3.92 ± 0.38	−1.57
Caudate R	4.08 ± 0.32	4.01 ± 0.32	−2.85*
Putamen L	6.55 ± 0.71	6.53 ± 0.70	−0.50
Putamen R	6.14 ± 0.57	6.06 ± 0.54	−2.26*
Thalamus L	7.03 ± 0.75	6.95 ± 0.76	−1.63
Thalamus R	7.36 ± 0.72	7.28 ± 0.77	−1.68
Pallidum L	2.00 ± 0.17	2.00 ± 0.17	−0.09
Pallidum R	1.58 ± 0.17	1.57 ± 0.16	−0.26
Accumbens L	0.66 ± 0.08	0.62 ± 0.09	−1.82
Accumbens R	0.78 ± 0.09	0.78 ± 0.09	−0.49
Ventricular structure	Baseline (mean ± sd)	Treatment (mean ± sd)	t-value
3rd ventricle	0.90 ± 0.23	0.95 ± 0.24	3.25#
4th ventricle	1.95 ± 0.56	2.07 ± 0.63	3.25#
Lateral ventricle L	5.63 ± 2.85	6.07 ± 2.78	2.87#
Lateral ventricle R	5.38 ± 2.05	5.76 ± 1.88	2.22*
Female-to-Male			
Subcortical structure	Baseline (mean ± sd)	Treatment (mean ± sd)	t-value
Hippocampus L	3.83 ± 0.29	3.83 ± 0.27	0.23
Hippocampus R	3.94 ± 0.30	3.95 ± 0.34	0.41
Amygdala L	1.48 ± 0.20	1.48 ± 0.20	−0.17
Amygdala R	1.48 ± 0.18	1.49 ± 0.18	1.17
Caudate L	3.64 ± 0.44	3.62 ± 0.44	−0.87
Caudate R	3.80 ± 0.46	3.78 ± 0.46	−0.77
Putamen L	5.94 ± 0.64	5.92 ± 0.67	−0.56
Putamen R	5.66 ± 0.62	5.62 ± 0.68	−0.73
Thalamus L	6.52 ± 0.72	6.58 ± 0.72	2.25*
Thalamus R	6.70 ± 0.81	6.73 ± 0.84	1.27
Pallidum L	1.84 ± 0.23	1.88 ± 0.25	2.32*
Pallidum R	1.43 ± 0.15	1.47 ± 0.17	2.17*
Accumbens L	0.63 ± 0.10	0.62 ± 0.11	−0.04
Accumbens R	0.72 ± 0.14	0.73 ± 0.14	1.36
Ventricular structure	Baseline (mean ± sd)	Treatment (mean ± sd)	t-value
3rd ventricle	0.75 ± 0.17	0.75 ± 0.17	0.28
4th ventricle	1.55 ± 0.40	1.52 ± 0.38	−2.20*
Lateral ventricle L	5.47 ± 3.26	5.53 ± 3.11	0.86
Lateral ventricle R	5.01 ± 3.48	5.01 ± 3.28	0.87

et al., 2000a, 2000b). But also non-classical G protein-coupled receptors are associated with E2 in this area (Hazell et al., 2009). Additionally, the large amount of androgen expressing cells in the hippocampus (Brown et al., 1995; Simerly et al., 1990) indicate an influence of testosterone in this brain structure.

The underlying mechanisms behind these structural changes are not entirely clear, but traditionally the effects of sex-steroid hormones on brain morphology are thought to be mediated via two distinct mechanisms. Action of steroid hormones can thus be dichotomized as organizational or activational (Cooke et al., 1998). While 'organizational effects' are present during early exposure to hormones prior to a critical period and mediate permanent and irreversible neuronal changes and sculpt the nervous system, 'activational effects' are characterized as transient changes in the fully developed brain, which only occur when the hormone is present, which is the case during acute hormone administration (Schulz et al., 2009; Sisk and Zehr, 2005). Although, Sisk and Zehr proposed that neural circuits are also organized during adolescence (Sisk and Zehr, 2005), it is believed that these activational effects, elicited by the exogenous hormone administration, are responsible for the observed changes in brain structures in our study.

Furthermore, sex-steroid hormones, such as testosterone, estradiol and progesterone exert their effects by acting via two distinct main modes of action. Rapid non-genomic effects or 'neuroactive effects' occur through binding to the cell membrane or membrane receptors, activating and modulating ligand-gated ion channels or G-protein coupled receptors (Rupprecht and Holsboer, 1999). They are able to alter membrane plasticity, permeability and excitability, neurotransmitter release, transmitter synthesis, or even the expression of receptors (Steiner et al., 2003). This is mainly done by modulating the function of certain enzymes, channels or even transmitter receptors. Progesterone, for example, is able to bind to the inhibitory $GABA_A$ receptor and enhances chloride current activated by GABA, exhibiting the same sedative and anticonvulsant effect as benzodiazepines (Follesa et al., 2000). On the other hand, genomic effects require a time period that lasts from minutes to hours and influence neuronal functioning by binding to intracellular receptors and regulate gene expression (Rupprecht and Holsboer, 1999). They can diffuse through the outer membrane and bind to specific steroid receptors in the cytoplasm and nucleus to promote or inhibit their transcription of specific genes in the nucleus (Steiner et al., 2003).

A simple model for the observed alterations in brain structure states that decreased volumes in MtFs are related to diminishing anabolic effects of testosterone by using anti-androgens and the putative adverse effects of estrogens on brain structure due to catechol estrogens (Zubiaurre-Elorza et al., 2014). Additionally, steroid hormones are crucial to maintain a specific neuroendocrine milieu, which leads to modulations in brain structure and function. Estradiol and progesterone for example, exert structural and functional trophic effects throughout life (Brinton et al., 2008; Wharton et al., 2012) by acting via the above mentioned receptors.

Our analysis suggests that progesterone is also highly involved in this process, as correlation analysis showed that reduction of progesterone predicted reduction in the right hippocampus and caudate. Here we showed that progesterone may imply a neuroprotective effect as a decrease in progesterone levels is correlated with decreases in gray matter volume. This is in line with prior studies indicating an involvement of progesterone in neuroprotection (Stein, 2013). In this regard, progesterone exerts pleiotropic effects and regulate genes involved in controlling inflammation, apoptosis and vascular remodelling (Anderson et al., 2011) and is thought to play a role in reparation, for example after brain injury (Sayeed and Stein, 2009; Stein, 2013). Studies already showed that it plays a central role in neuronal development during gestation (Baulieu and Robel, 1990; Schumacher et al., 1996). Furthermore, it leads to a reduction in neural degeneration and apoptosis (Drew and Chavis, 2000). Its treatment stimulates the synthesis of neurotrophic factors, such as BDNF (De Nicola et al., 2006; Stein and Wright, 2010).

Remarkably, the reduction in subcortical areas in MtF subjects led to simultaneous increases in the ventricular structures in MtFs. If and how these ventricular increases are related to the shrinkage of subcortical brain structures remains unclear. One possibility of increases in ventricular structures can be explained as a consequence of decreases in GM volume in nearby structure like in the hippocampus or the caudate. However, also white matter decreases may be responsible for the ventricular changes, as it was shown that sex-steroid hormones modulate white matter microstructure (Kranz et al., 2014; Rametti et al., 2012). Taken together, these findings point toward altered plasticity of structures in the adult human brain as effect of high-dose administration of cross-sex hormones. However, we cannot rule out that observed alterations in subcorti-

cal brain areas may in part be explained by regression toward the mean. Thus, interpretation of current results of hormonal effects must consider this effect. Furthermore, it has to be taken into consideration that the sample size of the control groups was essentially smaller compared to the FtM cohort which may also influence gained results.

Although, studies have already been conducted observing the influences of cross-sex hormonal treatment, our investigation showed changes in the hippocampus and the ventricles, which are corrected for multiple comparisons. We cannot generally support the notion that testosterone leads to masculinization of brain structures and estrogens and anti-androgen treatment to feminization of brain structures. However, we were able to corroborate prior findings that prolonged hormonal administration influences structures in the brain.

5. Conclusion

This study delivers evidence that cross-sex hormone therapy in transgender individuals leads to changes in subcortical brain areas. We showed that estradiol and anti-androgen treatment in MtF participants induced decreases in the hippocampus, while increases in the ventricles have been observed. While prior studies were limited by small sample sizes or presented uncorrected results, here we were able to show gray matter changes, corrected for multiple testing. Due to the use of the longitudinal pipeline implemented in FreeSurfer, our results can be seen as robust, as more sensitivity and specificity are provided by reducing the confounding effect of between-subject variability.

Conflict of interest

Without any relevance to this work, S. Kasper declares that he has received grant/research support from Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline, Lundbeck, Organon, Sepracor, and Servier; has served as a consultant or on advisory boards for AstraZeneca, Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline, Janssen, Lundbeck, Merck Sharp and Dome, Novartis, Organon, Pfizer, Schwabe, Sepracor, and Servier; and has served on speakers' bureaus for Angelini, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Janssen, Lundbeck, Pfizer, Pierre Fabre, Schwabe, Sepracor, and Servier. R. Lanzenberger has received travel grants and conference speaker honoraria from AstraZeneca, Roche, and Lundbeck A/S. G.S. Kranz has received travel grants from Roche, AOP Orphan Pharmaceuticals, and Pfizer. All other authors report no biomedical financial interests or potential conflicts of interest.

Contributors

R.L. and S.K. designed the study and R.Se., G.S.K., A.Ha. and R.L. wrote the manuscript. Authors A.K. and C.K. managed the literature searches and analyses. C.W., R.Sl., A.Hu. and M.W. performed the measurements. Authors R.Se., A.Ha., G.S.K. and S.G. undertook the statistical analysis, and R.Se. wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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