Research Paper

Changes to hypothalamic volume and associated subunits during gender-affirming hormone therapy

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Background: Among its pleiotropic properties, gender-affirming hormone therapy (GHT) affects regional brain volumes. The hypothalamus, which regulates neuroendocrine function and associated emotional and cognitive processes, is an intuitive target for probing GHT effects. We sought to assess changes to hypothalamus and hypothalamic subunit volumes after GHT, thereby honouring the region's anatomical and functional heterogeneity. **Methods:** Individuals with gender dysphoria and cisgender controls underwent 2 MRI measurements, with a median interval of 145 days (interquartile range [IQR] 128.25–169.75 d, mean 164.94 d) between the first and second MRI. Transgender women (TW) and transgender men (TM) underwent the first MRI before GHT and the second MRI after approximately 4.5 months of GHT, which comprised estrogen and anti-androgen therapy in TW or testosterone therapy in TM. Hypothalamic volumes were segmented using FreeSurfer, and effects of GHT were tested using repeated-measures analysis of covariance. **Results:** The final sample included 106 participants: 38 TM, 15 TW, 32 cisgender women (CW) and 21 cisgender men (CM). Our analyses revealed group × time interaction effects for total, left and right hypothalamus volume, and for several subunits (left and right inferior tubular, left superior tubular, right anterior inferior, right anterior superior, all $p_{corr} < 0.01$). In TW, volumes decreased between the first and second MRI in these regions (all $p_{corr} \leq 0.01$), and the change from the first to second MRI in TW differed significantly from that in CM and CW in several subunits ($p_{corr} < 0.05$). **Limitations:** We did not address the influence of transition-related psychological and behavioural changes. **Conclusion:** Our results suggest a subunit-specific effect of GHT on hypothalamus volumes in TW. This finding is in accordance with previous reports of positive and negative effects of androgens and estrogens, respectively, on cerebral volumes.

Introduction

As the neuroendocrine control centre of the human body, the hypothalamus is both functionally and anatomically complex.[1] Various nuclei facilitate a range of processes[1,2] from body temperature regulation[3] and energy homeostasis[4] to sexual[5] and aggressive behaviour.[6] Sex hormones influence brain development throughout life, from the prenatal to the adult brain, by exerting "organizational" and "activational" effects, respectively.[7] The hypothalamus, which exhibits high concentrations of estrogen and androgen receptors[8] and is a central regulator of feedback loops controlling sex hormone homeostasis,[2] may be particularly susceptible to the effects of sex hormones. In fact, the third interstitial nucleus of the anterior hypothalamus (INAH-3) displays the largest sex-related volumetric difference in the human brain,[9] and volumetric sex differences have been observed in various hypothalamic substructures.[10–13] Changes to hypothalamic volume have been shown under oral contraception[14] and analyzed across the menstrual cycle.[14,15] Tight interplay between the hypothalamus and sex hormones makes the hypothalamus a target for studies on disorders, such as depression, in which sex hormones mediate risk.[16–18]

Gender-affirming hormone therapy (GHT)[19] allows for investigation of the effects of highly dosed sex hormones on the human brain. The impact of GHT on brain volumes, including that of the hypothalamus, has been assessed previously using magnetic resonance imaging (MRI) in individuals with gender dysphoria (GD). Testosterone therapy in transgender men (TM) led to an increase in total brain volume,[20] total grey matter volume[21,22] and cortical thickness,[21–23] but a decrease in subcortical volumes.[24] In contrast, anti-androgen and estrogen

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therapy in transgender women (TW) was associated with a decline in total brain volume,[20] total grey matter volume[21,22] and volume of various subcortical regions.[20,22,25] Regarding the hypothalamus specifically, a volumetric reduction in TW was shown under GHT. However, existing studies have had small sample sizes, assessed only total hypothalamic volume, and lacked information on the effects of GHT on hypothalamic substructures. Thus, existing studies do not reflect the anatomic and functional heterogeneity of the region.[1]

Here we examined hypothalamus volume, including volume of its subunits, before and after approximately 4.5 months of GHT in individuals with GD. We expected changes in accordance with previous volumetric studies, more specifically volumetric increases and decreases after testosterone and anti-androgen and estrogen treatment, respectively. Based on the region's heterogeneity,[1] we expected subunit-specific effects.

Methods

Sample

Data analyzed here were gleaned from 2 clinical trials by our group, the results of which have been published previously.[24–31] We considered data from 125 right-handed individuals who underwent 2 MRI measurements in a longitudinal design. Nineteen data sets were excluded because of poor MRI data segmentation quality (1 individual) or missing blood hormone levels (18 individuals).

Transgender individuals were recruited from the transgender outpatient unit at the Department of Obstetrics and Gynecology, Unit for Gender Identity Disorder, Medical University of Vienna. They fulfilled *Diagnostic and Statistical Manual of Mental Disorders* (DSM)-IV criteria for gender identity disorder or DSM-5 criteria for GD. Cisgender individuals were recruited via online media and flyers on designated message boards throughout Vienna. Participants with presence or history of a physical, neurologic, or psychiatric disorder (cisgender individuals) or a DSM-IV or DSM-5 Axis-I comorbidity (individuals with GD, excluding affective and anxiety disorders), abnormal blood values or abnormalities in clinical examinations were excluded from the study. Additionally, pregnancy, breastfeeding, substance misuse (except tobacco consumption), steroid hormone therapy within 6 months before the start of the study (with the exception of progestin-only oral contraception for cessation of menstruation in a subset of TM participants) and any MRI contraindications were set as exclusion criteria.

Study design

Both clinical trials were conducted as longitudinal single-centre studies. Two MRI measurements were performed in all participants, with a median interval of 145 days (interquartile range [IQR] 128.25–169.75 d, mean 164.94 d) between the first and second MRI. The median intervals by gender were as follows: median 134.5 (IQR 126–152.25, mean 150.29) days in TM, median 149 (IQR 126–178.5, mean 155.3) days in TW, median 147 (IQR 139.75–170.5, mean 163.81) days in cisgender women (CW) and median 146 (IQR 132–247, mean 200.05) days in cisgender men (CM). Gender-affirming hormone therapy was initiated in transgender individuals immediately after the first MRI and was performed according to standard protocols of the Department of Obstetrics and Gynecology, Unit of Gender Identity Disorder of the Medical University of Vienna.

Both clinical trials were approved by the Ethics Committee of the Medical University of Vienna (1104/2015; 644/2010) and registered at ClinicalTrials.gov (NCT02715232; NCT01292785). Each participant gave written informed consent, was insured and received financial compensation for their participation. All study procedures were in accordance with the declaration of Helsinki and latest revisions as well as the *Good Scientific Practice* guidelines of the Medical University of Vienna.

Gender affirming hormone therapy

Transgender men received either 1000 mg testosterone undecanoate every 8–12 weeks (intramuscular) or 50 mg testosterone cream or gel daily (transdermal). Additionally, in some cases 75 µg desogestrel daily (oral) or 10–15 mg lynestrenol daily (oral) was administered for cessation of menstruation, and in some cases this treatment was initiated before the first MRI. Transgender women received 25–50 mg cyproterone acetate daily (oral) and either 100 µg estradiol via a transdermal therapeutic system twice a week, 4 mg estradiol daily (oral) or 0.75–3 mg estradiol daily (transdermal). If extensive hair loss occurred, 2.5 mg finasteride (oral) every other day was prescribed. Some participants additionally received 100–200 mg progesterone daily (oral). Both TM and TW may also have received 100 µg triptorelin acetate daily or 4.12 mg triptorelin acetate monthly (subcutaneous or intramuscular) or 11.25 mg leuprorelin acetate every 3 months (subcutaneous).

Blood hormone sampling

Blood draw for hormone sampling was performed at each MRI measurement in all participants. Plasma levels of dehydroepiandrosterone sulfate (DHEAS), 17β-estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, sex hormone–binding globulin (SHBG) and testosterone were analyzed by the Department of Laboratory Medicine, Medical University of Vienna.

MRI data acquisition

The MRI measurements were obtained using either Siemens MAGNETOM Prisma or Siemens TIM Trio scanners with 64-channel or 32-channel head coils, respectively. T_1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequences (Siemens MAGNETOM Prisma: echo time 2.91 ms, repetition time 2000 ms, inversion time 900 ms, $\alpha = 9°$, 192 slices, matrix 240 × 256, voxel size 1 × 1 × 1 mm^3; Siemens TIM Trio: echo time 4.21 ms, repetition time 2300 ms, inversion time 900 ms, $\alpha = 9°$, 160 slices, matrix 240 × 256, voxel size 1.1 × 1 × 1 mm^3; total acquisition time 7 min, 46 s) were used.

MRI data processing

A visual quality check of MRI data was performed. Participants were processed with the standard FreeSurfer software suite pipeline, version 7.2 (http://surfer.nmr.mgh.harvard.edu/),[32,33] followed by the longitudinal stream.[34] Subsequently, the hypothalamus and its subunits were segmented using the respective segmentation tool distributed with FreeSurfer.[35] With this tool, hypothalamic subnuclei are assigned to 5 hypothalamic subunits per side, classified according to Makris and colleagues[36] and Bocchetta and colleagues.[37] See Appendix 1, Figure S1, available at www.jpn.ca/lookup/doi/10.1503/jpn.230017/tab-related-content, for visual representation of segmentation. Automated segmentation was checked visually by a trained neuroscientist. LongCombat was used to correct for means and variances of the residuals across different scanners in this longitudinal setting.[38] Using this method, the variables of interest and covariates were harmonized for different scanners, while group and time interactions were set to be maintained.

Statistical analysis

Statistical analysis was performed using SPSS version 25 for Windows (SPSS Inc.). We tested for group differences in age and scan interval using 2-sample *t* tests. Repeated-measures analysis of covariance (ANCOVA) was used to probe for changes to hypothalamic volumes under GHT. Group (CW, TM, CM, TW) was used as a between-subjects factor, whereas time (first MRI, second MRI) was included as the within-subjects factor. The group × time interaction was examined in a stepwise approach. We performed a separate analysis of the total hypothalamic volume; if significant, each side was analyzed, and if significant again, each subunit on the respective side was tested. To correct for varying hormone therapy regimens within transgender groups we included DHEAS, SHBG, FSH, LH, progesterone, estradiol and testosterone levels as covariates to adjust for interindividual differences. To reduce the dimensionality of these values, we applied a principal component analysis (PCA), and the first 2 components explaining more than 98% of the total variance were integrated into our statistical model as covariates. The total intracranial volume was also included as a covariate.[9] The Bonferroni method was used to correct for multiple comparisons in a level-wise approach. In addition, analyses were repeated including scan intervals as covariates based on a statistically significant difference between TM and CM.

Results

Our study sample included 106 participants: 32 CW (mean age ± standard deviation [SD] 24.81 ± 6.04 yr), 38 TM (26.11 ± 6.72 yr), 21 CM (26.48 ± 6.59 yr) and 15 TW (25.73 ± 3.75 yr). Demographic data and hormone levels are shown in Table 1. Groups did not differ significantly in age. The TM group differed significantly from the CM group in scan interval ($p = 0.02$, uncorrected).

Repeated-measures ANCOVA revealed a group × time interaction effect for total hypothalamus volume ($F = 6.28$, $p_{corr} < 0.01$). Significant group × time interaction effects were also found for the left ($F = 6.40$, $p_{corr} < 0.01$) and right ($F = 6.08$, $p_{corr} < 0.01$) hypothalamus individually. In the single subunits, significant interaction effects were found in the left inferior tubular subunit ($F = 7.27$, $p_{corr} < 0.01$), right inferior tubular subunit ($F = 7.29$, $p_{corr} < 0.01$), left superior tubular subunit ($F = 6.69$, $p_{corr} < 0.01$), right anterior superior subunit ($F = 7.03$, $p_{corr} < 0.01$) and right anterior inferior subunit ($F = 7.39$, $p_{corr} < 0.01$). In TW, change over time from the first MRI to the

Table 1: Age and hormone values*

Value, mean ± SD	CW *n* = 32		TM *n* = 38		CM *n* = 21		TW *n* = 15	
	MRI1	MRI2	MRI1	MRI2	MRI1	MRI2	MRI1	MRI2
DHEAS, µg/mL	2.85 ± 0.84	2.99 ± 0.98	2.88 ± 1.64	3.17 ± 1.55	3.62 ± 1.52	3.58 ± 1.16	3.33 ± 1.06	3.56 ± 1.2
Estradiol, pg/mL	107.42 ± 86.15	109.13 ± 77.59	119.41 ± 76.95	70.45 ± 54.84	44.40 ± 70.38	29.05 ± 14.57	35.65 ± 15.91	232.91 ± 261.63
FSH, mIU/mL	4.98 ± 2.34	4.83 ± 2.66	4.30 ± 2.27	5.26 ± 3.1	3.9 ± 1.65	4.12 ± 1.46	4.17 ± 2.13	1.35 ± 2.4
LH, mIU/mL	13.31 ± 18.4	9.18 ± 7.58	8.58 ± 5.8	8.02 ± 8.25	6.56 ± 2.5	6.56 ± 2.42	6.02 ± 2.37	1.42 ± 2.16
Progesterone, ng/mL	2.80 ± 4.04	4.01 ± 4.87	5.73 ± 6.78	1.39 ± 2.55	1.65 ± 4.69	0.68 ± 0.65	0.49 ± 0.29	0.80 ± 0.73
SHBG, nmol/L	68.89 ± 36.85	68.51 ± 36.07	66.75 ± 37.15	43.89 ± 30.09	48.62 ± 29.7	39.57 ± 15.25	44.31 ± 17.82	61.01 ± 34.72
Testosterone, ng/mL	0.38 ± 0.15	0.54 ± 0.88	0.38 ± 0.17	3.87 ± 2.44	4.99 ± 2.59	5.14 ± 1.78	4.2 ± 2.67	1.43 ± 2.40
Age, yr†	24.81 ± 6.04		26.11 ± 6.72		26.48 ± 6.59		25.73 ± 3.75	

CM = cisgender men; CW = cisgender women; DHEAS = dehydroepiandrosterone sulfate; FSH = follicle-stimulating hormone; GHT = gender-affirming hormone therapy; LH = luteinizing hormone; SHBG = sex hormone-binding globulin; TM = transgender men; TW = transgender women.
*Data are given from baseline (MRI1) and after approximately 4.5 months (MRI2) of GHT (in TW and TM). Age is given from MRI1.

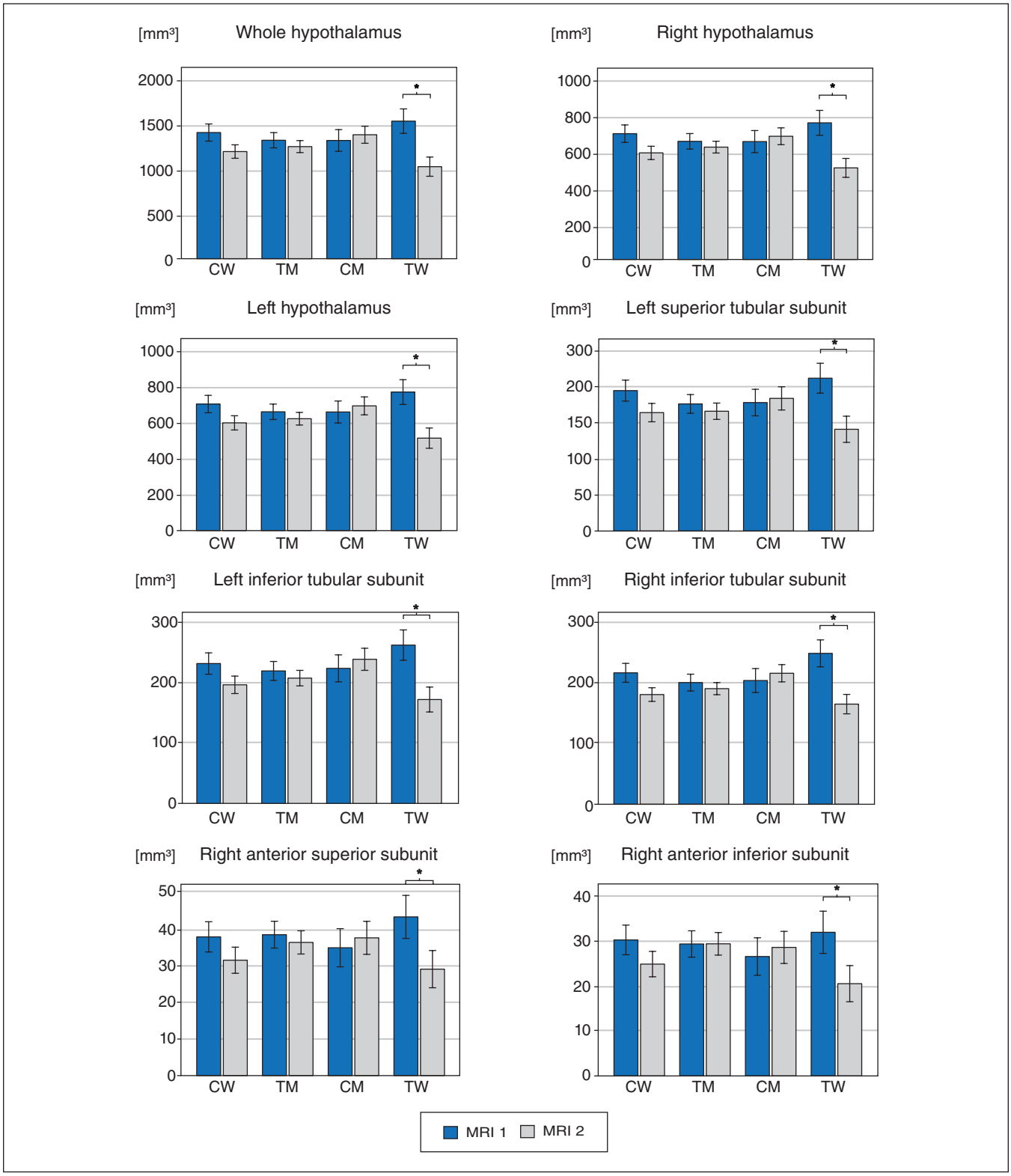

Figure 1: Decreased volumes of the hypothalamus and associated subunits in transgender women (TW) after gender-affirming hormone therapy (GHT). Post hoc comparisons between baseline MRI (MRI 1) and after approximately 4.5 months (MRI 2) of GHT in TW and transgender men (TM; no therapy in cisgender men [CM] and cisgender women [CW]) show significant volumetric reduction, in cubic millimetres, in the whole hypothalamus; in the left and right hypothalamus separately; and in the left and right inferior tubular subunit, right anterior inferior subunit, right anterior superior subunit, left superior tubular subunit in TW. *$p_{corr} \leq 0.01$.

second MRI differed significantly from that in CM and CW in total, left and right hypothalamus volume as well as in the subunits mentioned previously (all $p_{corr} < 0.05$), with the exception of the left superior tubular subunit and right anterior inferior subunit, which showed a trend toward significance in the comparison of TW and CW ($p_{corr} = 0.05$). Post hoc comparisons showed a significant volume decrease for TW in each of the regions mentioned above (all $p_{corr} \leq 0.01$; Figure 1 and Table 2). In CW, quantitatively smaller hypothalamus volumes were observed at the second MRI compared with the first MRI; however, these differences did not reach significance. Also, in TM and CM, no significant differences between the 2 MRIs were detected.

Repeating the analysis including scan intervals as covariates did not affect significance (Appendix 1).

Discussion

We assessed changes to hypothalamus and hypothalamic subunit volumes after approximately 4.5 months of GHT in individuals with GD (TM, TW) and untreated cisgender controls (CM, CW). We detected significant volume reductions in the total hypothalamus, in both the left and right hypothalamus separately and in 5 of 10 hypothalamic subunits (inferior tubular subunit bilaterally, left superior tubular subunit, right anterior superior subunit and right anterior inferior subunit) in TW receiving estrogen and anti-androgen therapy. No changes were detected after GHT in TM. These findings are in accordance with previous studies showing cortical and subcortical volume reduction in TW receiving GHT.[20,22,25] A previous study of 6 TW and 8 TM[20] specifically reported volume reduction in the hypothalamus, which we have now confirmed in a substantially larger sample. In addition, the subunit-specific nature of the effects we detected are in accordance with the region's anatomic and functional heterogeneity.[1]

Our study suggests an effect of GHT on volumes of and within the hypothalamus. The hypothalamus has been implicated in various psychiatric disorders, such as depression,[17,18] for which sex hormones mediate risk.[16] Elucidating how sex hormones affect the hypothalamus may foster understanding of how they modulate this risk on a neurobiological level.

Our results in TW may be facilitated by increasing estrogen or decreasing androgen levels. Negative effects of estrogen on brain volumes, including the hypothalamus, have been reported extensively in animals[10,39,40] and humans. In humans, they are implicated via sex differences showing smaller hypothalamus volumes in women,[9] associations with the menstrual cycle with negative effects in the periovulatory phase,[15] and smaller volumes with administration of exogenous estrogens (e.g., via oral contraception).[14] In vitro evidence suggests that estradiol exerts neurotoxic effects.[39] On the other hand, some androgens may have anabolic and/or anticatabolic effects.[19,22,41] In fact, we previously showed a decrease in mean diffusivity in TM who received GHT with testosterone, a change interpreted as indicating increased microstructural plasticity in this region.[27] For example, while gonadectomized male mice under estrogen and progesterone treatment showed significant volume reductions in hypothalamic nuclei, mice receiving estrogen and progesterone treatment without gonadectomy did not.[42] Thus, the presence of androgens antagonized the catabolic effects of estrogen.[42] However, effects may vary among androgens as well. It was suggested that 5α-dihydrotestosterone (DHT) may have an anticatabolic effect while testosterone, like estrogen, may have neurotoxic properties in the hypothalamus.[39] Thus, though our design could not discriminate between the effect of increasing estrogen or decreasing androgens, preclinical evidence speaks to the latter, specifically of falling DHT levels. Testosterone is aromatized to DHT, including within the hypothalamus.[39] In our study, the lack of an effect in TM, who received exogenous testosterone treatment, speaks to balance between the antagonistic effects of DHT and testosterone.

We performed volumetric analyses using MRI, thus, discussion of potential histochemical correlates is theoretical. However, preclinical studies provide potentially relevant interpretations. In vitro, estrogen increases hypothalamic glial activity, an index of neurotoxicity.[39] In humans in vivo, falling estrogen levels after discontinuation of oral contraception have been shown to increase diffusion and exert metabolic effects, as measured with MRI and magnetic resonance spectroscopy.[43] Increased diffusivity may reflect microstructural reorganization such as changes to neuron–glial interaction.[44] The observed decrease in choline, which is present in cell membranes,[45] has been postulated to reflect altered cell turnover.[43] Thus, on a histochemical level, our findings may be associated with cellular remodelling.

We primarily detected volume decreases in anterior and tubular regions of the hypothalamus. These subunits harbour hypothalamic nuclei involved in stress, arousal, sexual behaviour, aggression and sleep.[2] Gender-affirming hormone therapy has been associated with changes in the biochemical and/or behavioural correlates of stress,[46] sex[47] and potentially aggression.[48–50] Whether the volume reductions we observed are functionally linked to these clinical and behavioural correlates remains to be elucidated in future studies.

Table 2: Hypothalamic and associated subunit volumes in TW at baseline and after approximately 4.5 months of GHT

	Volume, mean ± SE, mm³*	
Brain region	MRI 1	MRI 2
Whole hypothalamus	1513.79 ± 67.84	1012.64 ± 53.26
Left hypothalamus	762.05 ± 34.33	508.70 ± 28.05
Right hypothalamus	751.74 ± 34.46	503.94 ± 26.23
Left inferior tubular subunit	261.24 ± 12.64	171.45 ± 10.36
Right inferior tubular subunit	249.04 ± 11.21	165.29 ± 8.08
Left superior tubular subunit	210.19 ± 10.37	139.93 ± 9.07
Right anterior superior subunit	43.65 ± 2.98	29.28 ± 2.58
Right anterior inferior subunit	31.61 ± 2.34	20.30 ± 2.01

GHT = gender-affirming hormone therapy; SE = standard error; TW = transgender women.
*Corrected for covariates using longCombat.[38]

The reported effects were observed during GHT and thus suggest, but do not prove, a hormone-specific effect. This particularly holds true because we included hormone values as covariates in our model in order to address interindividual differences in GHT. On the other hand, our observations in TW may be associated with other hormonal, neurobiological or behavioural factors that change over the course of GHT, for which we could not statistically correct in our study. The hypothalamus is involved in various cognitive and emotional processes,[1,5,6] which may also change during transition.

Limitations

The following limitations should be considered when interpreting our results. The hypothalamus is an anatomically small structure and exhibits low image contrast in MRI. However, we used an automated tool within FreeSurfer that performs segmentation of the whole hypothalamus as well as its subunits in T_1-weighted brain MRIs accurately and robustly.[35] Moreover, the hypothalamus is located adjacent to the third ventricle.[1] Previous studies have shown increased ventricle volumes in TW after 4 months of estrogen and antiandrogen therapy.[20,22,25] We sought to account for this effect by including total intracranial volume into our analysis. Furthermore, the data analyzed here were acquired on 2 different 3 T MRI devices. Though we addressed this issue by correcting for between-scanner differences using longCombat,[38] we cannot definitively exclude influential factors. As discussed, though our results suggest hormone effects, we cannot exclude other confounding factors that occur over the course of transition. Effects resulting from differences in GHT regimens were addressed by including hormone levels as covariates in our statistical model.

Conclusion

We detected volumetric reduction within the hypothalamus, driven by 5 specific subunits, in TW under GHT. The subunit specificity of these results is in accordance with the anatomic and functional heterogeneity[1,35] of the hypothalamus. These findings suggest a negative effect of estradiol and/or a positive or anticatabolic effect of testosterone and confirm the findings of previous, substantially smaller studies in TW.[20] Considering the region's involvement in psychiatric pathology, examining how sex hormones influence the hypothalamus may promote understanding of how they affect this risk on a neurobiological level. Further studies are needed to elucidate underlying histochemical mechanisms and their functional relevance.

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Contributors: G.S. Kranz and R. Lanzenberger designed the study. U. Kaufmann, P.A. Handschuh, M Klöbl, C. Schmidt, G.M. Godbersen, R. Seiger and P. Baldinger-Melich acquired the data, which M.E. Konadu, M.B. Reed, B. Spurny-Dworak, E. Briem and M. Spies analyzed. M.E. Konadu and M. Spies wrote the article. All of the authors revised it critically for important intellectual content, gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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Data sharing: For reasons of data protection, the data are available on reasonable request from the corresponding author.

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