Effects of gender-affirming hormone therapy on gray matter density, microstructure and monoamine oxidase A levels in transgender subjects

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Highlights

- Gender-affirming hormone therapy led to significant changes in gray matter density and microstructure in various brain regions

- Gray matter changes found after gender-affirming hormone therapy were not reflected by monoamone oxidase A density changes in the brain.

- Masculinizing gender-affirming hormone therapy resulted in increased sexual desire in transgender men.

Abstract

34 MAO-A catalyzes the oxidative degradation of monoamines and is thus implicated in sex-

35 specific neuroplastic processes that influence gray matter (GM) density (GMD) and

36 microstructure (GMM). Given the exact monitoring of plasma hormone levels and sex steroid

37 intake, transgender individuals undergoing gender-affirming hormone therapy (GHT) represent

38 a valuable cohort to potentially investigate sex steroid-induced changes of GM and

39 concomitant MAO-A density. Here, we investigated the effects of long-term GHT over a median

40 time period of 4.5 months on GMD and GMM as well as MAO-A distribution volume. To this

41 end, 20 cisgender women, 11 cisgender men, 20 transgender women and 10 transgender men

42 underwent two MRI scans in a longitudinal design. PET scans using [^{11}C]harmine were

43 performed before each MRI session in a subset of 35 individuals. GM changes determined by

44 diffusion weighted imaging (DWI) metrics for GMM and voxel based morphometry (VBM) for

45 GMD were estimated using repeated measures ANOVA. Regions showing significant changes

46 of both GMM and GMD were used for the subsequent analysis of MAO-A density. These

47 involved the fusiform gyrus, rolandic operculum, inferior occipital cortex, middle and anterior

48 cingulum, bilateral insula, cerebellum and the lingual gyrus (post-hoc tests: $p_{FWE+Bonferroni}$ <

49 0.025). In terms of MAO-A distribution volume, no significant effects were found. Additionally,

50 the sexual desire inventory (SDI) was applied to assess GHT-induced changes in sexual

51 desire, showing an increase of SDI scores among transmen. Changes in the GMD of the

52 bilateral insula showed a moderate correlation to SDI scores (rho = - 0.62, $p_{Bonferroni}$ = 0.047).

53 The present results are indicative of a reliable influence of gender-affirming hormone therapy

54 on 1) GMD and GMM following an interregional pattern and 2) sexual desire specifically among

55 transmen.

56

Introduction

As pointed out in several reviews, meta- and mega-analyses that have been published over the last decades, a multitude of studies on sex-specific differences in brain structure point towards significant effects of sex steroids in terms of global, inter- and intra-regional brain morphology (1-5). On average, female brains are smaller than male ones (6). Significant differences between male and female brains were also found regarding gray (GM) and white matter (WM) volumes (7, 8). Nevertheless, some studies failed to detect such differences in GM volume on a global scale, despite adjustment for total brain size (9). Both region-of-interest (ROI)-based approaches as well as voxel-wise analyses allow for the examination and classification of highly specific cortical and subcortical regions, shedding light on a vast number of potential regional and/or local sex differences (3-5). However, a closer look to the current evidence reveals some inconsistent results, questioning the theory of a sexually dimorphic brain and pointing towards a lack of features found reliably at one end of the continuum between typically female and male brains (10).

Even though previous literature has reported conflicting results, it is known that sex hormones are centrally involved in the biological mechanisms that drive sex-specific differences in brain structure. Hence, ongoing research focusing on the interaction of cycling sex steroid levels with components of the central nervous system is essential. Sex steroids are known to have both transcriptional as well as non-genomic effects (11-13), modifying the brain throughout the lifespan (14-17). Thus, distributional differences in sex hormone receptors across the brain (18-20) as well as sex-specific differences in axonal characteristics (21), neuroplasticity (22) and neurogenesis (23) can be found. Additionally, sex steroids are known to directly interact with various neurotransmitters (24-26) and their metabolism, e.g., by modulating the activity of cerebral MAO-A activity. This has already been shown in a number of animal models (27-30) and selected human studies (31), however *in vivo* imaging data acquired via positron emission tomography (PET) remains scarce (32-34) and studies investigating the direct effects of exogenously administered sex hormones on cerebral MAO-A are specifically

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84   underrepresented (35). A further gap in the literature that needs to be addressed is given by

85   studies investigating the effect of *MAOA* gene polymorphisms that were shown to interact with

86   GM changes – despite knowledge on the influence of MAOA VNTR polymorphism on brain

87   structure volumes (36), cortical thickness (37) and gray matter (38), the association of MAO-A

88   protein expression as measured in human *in vivo* studies and GM is insufficiently investigated.

89   Taking into account the relevance of MAO-A density and subsequent neurotransmitter

90   metabolism on neuroplastic effects (39-41) and thus GM characteristics in the brain, the link

91   between MAO-A distribution and GM needs to be explored with multimodal imaging

92   techniques. Taking into account the additional effect of sex steroids on both MAO-A density

93   (32-34) as well as GM (7, 8), sex-specific interrelations of MAO-A density and GM might be of

94   interest.

95   Individuals with gender dysphoria (GD) receiving gender-affirming hormone therapy (GHT)

96   represent a unique cohort to investigate the direct effects of sex hormones on human brain

97   structure. As described in the 5th edition of the Diagnostic and Statistical Manual of Mental

98   Disorders (DSM-V), GD is defined as an incongruence between the biological sex assigned at

99   birth and the experienced gender later in life (42). GHT basically involves the long-term

100   administration of an estrogen and a type of androgen blocker in transgender women (TW),

101   whereas the masculinization of transgender men (TM) can be supported by testosterone

102   treatment (43). As summarized by Kranz and colleagues, structural changes of the brain were

103   observed after GHT in a wide range of studies (44), with effects of testosterone treatment on

104   total brain volume (45, 46), GM and WM density (47-49) as well as cortical thickness, showing

105   controversial results in the latter (47, 50). In terms of estrogen and antiandrogen treatment, a

106   decrease in cortical thickness (47), total brain (45) as well as total GM and WM volume (46)

107   was shown. Changes in diffusivity as it was found after sex steroid treatment both in TM and

108   TW might be traced back to changes in the organizational properties of neural fibers (e.g.,

109   axon diameter, fiber density, myelin staining) (44). On a molecular level, high-dose

110   testosterone treatment was shown to influence the distribution volume (V_T) of MAO-A in

111   transgender individuals (33). The structural and molecular effects of sex steroids on the brain

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112   are also reflected by behavioralchanges, e.g., in terms of sexual behavior. In a cross-sectional

113   multi-centered transgender study, Garz et al. could show that masculinizing GHT was

114   associated with increased sexual desire in transmen (51). This is in line with the findings of

115   Defreyne et al. (52), who reported a long-lasting increase of sexual desire as measured by the

116   sexual desire inventory (SDI) in TM after GHT, whereas in TW, multiple SDI subscores

117   decreased during the first three months of GHT.

118   In this longitudinal study, we aimed to investigate changes to brain morphology and

119   microstructure and further explore the corresponding effects on MAO-A binding in transgender

120   individuals after long-term GHT. To reveal alterations in GM density (GMD) and microstructure

121   (GMM) via MRI, voxel-based morphometry (VBM) and diffusion weighted imaging (DWI) were

122   applied. In a subset of participants, PET imaging using [^{11}C]harmine was conducted to uncover

123   the effects of sex steroid treatment on cerebral MAO-A density and to elucidate potential

124   interrelations between hormonally induced GM changes and alterations in MAO-A V_T. In

125   addition, we aimed to reveal the effect of GHT on sexual desire in both transmen and

126   transwomen.

Methods

128   This study was performed according to the Declaration of Helsinki, taking into account all

129   current revisions and good scientific practice guidelines of the Medical University of Vienna.

130   The study protocol was approved by the Ethics Committee of the Medical University of Vienna

131   (EC number 1104/2015) and registered at clinicaltrials.gov (NCT02715232).

Participants

133   Twenty cisgender women (CW), 11 cisgender men (CM), 20 TW and 10 TM men were enrolled

134   in the study. Transgender subjects were recruited at the Unit for Gender Identity Disorder at

135   the Department of Obstetrics & Gynecology, Division of Gynecologic Endocrinology and

136   Reproductive Medicine, Medical University of Vienna, Austria. Control subjects were recruited

137   via designated message boards at the Medical University of Vienna and at other universities

138   in Vienna as well as via social media platforms. CW and CM were age and education level

139   matched to TW and TM, respectively. Importantly, for transgender individuals, inclusion criteria

140   comprised a diagnosis of gender dysphoria as defined in the Diagnostic and Statistical Manual

141   for Mental Disorders), version 5 (DSM-5: 302.85) or the International Classification of

142   Diseases, version 10 (ICD-10: F64.1). Moreover, the absence of severe psychiatric, neurologic

143   or internal comorbidities was ensured in case of study enrollment based on medical history,

144   physical examination, electrocardiogram, laboratory screening and structural clinical interview

145   (SCID) for DSM-V. All subjects gave written informed consent to take part in this study and

146   received financial compensation for their participation. Subjects were excluded in case of major

147   neurological or internal illnesses, pregnancy, current substance abuse (excluding smoking),

148   any kind of pathologic laboratory values, GHT or any other treatment with exogenous sex

149   hormones within 6 months prior to study enrollment, MRI contraindications, current or past

150   substance-related disorder or non-compliance (all subjects), any kind of current or prior

151   psychiatric diagnosis (CW and CM) or severe DSM-V comorbidities (TW and TM) as well as

152   current (TW and TM) or prior (CW and CM) treatment with psychotropic agents.

Study design

154   All subjects underwent two MRI scans separated by a median time period of 4.5 months

155   following a longitudinal study design. Each MRI session included structural (T1- and diffusion-

156   weighted), task-based and resting-state MRI as well as MR spectroscopy. For the current

157   analyses, only structural MRI data was used. Thirty-five subjects (9 CW, 8 CM, 7 TW and 11

158   TM), underwent PET scan(s) that were performed before MRI acquisition using the radiotracer

159   [^{11}C]harmine. After the baseline imaging sessions, transgender subjects received GHT.

160   Plasma hormone levels were assessed via blood draw right before or after each baseline and

161   follow-up imaging session. In addition, participants underwent the sexual desire inventory

162   2(SDI-2) to assess potential effects of GHT on participants' interest in sexual activity.

Medication

The following GHT protocol was applied at the Department of Obstetrics & Gynecology, Division of Gynecologic Endocrinology and Reproductive Medicine, Medical University of Vienna, Austria: TM were treated with either 1000 mg testosterone undecanoat every 8-16 weeks (Nebido 1000 mg / 4 ml i.m.), or up to 50 mg testosterone daily (Testogel 50 mg/day or Testavan 23 mg/pump 1-2 pumps/day or testosterone crème as a magistral formula 12,5 mg/pump 3-4 pumps/day transdermally). If menstruation was still observed, transgender male participants received either lynestrenol (Orgametril 5 mg 2-3 tablets/day p.o.) or desogestrel (Moniq Gynial or Cerazette 0.075 mg/day p.o.). TW received cyproterone acetate daily (Androcur 50 mg/day) and either estradiol transdermally (Estramon 100 µg transdermal patch 2x/week or Estrogel gel 0.75-1.5 mg/day transdermally) or oral estradiol (Estrofem 4 mg/day p.o.). In case of extensive hair loss, an alpha-5-reductase-inhibitor was prescribed (Finasterid 2.5 mg/48h Actavis/Arcana/Aurobindo p.o.). Furthermore, selected participants were treated with GnRH-analogues like triptorelin (decapeptyl 0.1 mg/day subcutaneously).

Sex steroid monitoring

Levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, estradiol, testosterone, sex hormone binding globulin (SHBG) and dehydroepiandrosterone sulfate were assessed via blood analysis at baseline and after a median time period of 4.5 months involving GHT in transgender subjects and no treatment in cisgender controls.

Magnetic resonance imaging acquisition

All MRI data were recorded using a Siemens Prisma 3T scanner and a 64-channel head coil. A whole-brain T1-weighted scan was acquired with the following parameters: TE / TR = 2.91 / 2000 ms; inversion time = 900 ms; flip angle = 9°; matrix = 240 x 256, 176 slices; voxel size = 1.0 mm^3; acquisition time = 7:59 min. Diffusion weighted images contained 64 diffusion encoded images, with a b-value of 800 s/mm^2 along with 7 non-diffusion (b=0) images (TE / TR=76 / 9400 ms, slice thickness = 1.6 mm, resolution=1.6 x 1.6 mm, matrix size=128 x 128 x 75, flip angle = 90°, acquisition time = 11:54 min). Subjects were instructed to stay awake

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190 with eyes open and keep their head as still as possible during the entire scan to minimize

191 movement artifacts.

Positron emission tomography

193 Synthesis and quality control of [^{11}C]harmine, (7-[^{11}C]methoxy-1-methyl-9H-pyrido[3,4-

194 b]indole) were performed as previously published (53). All PET scans were performed using a

195 GE Advance full-ring scanner (General Electric Medical Systems, Milwaukee, WI, USA) at the

196 Department of Biomedical Imaging and Image-guided Therapy, Division of Nuclear Medicine,

197 Medical University of Vienna (54-56). A 5 min transmission scan was done at the beginning of

198 the scan for tissue attenuation correction. Data acquisition started simultaneously with an

199 intravenous bolus injection of [^{11}C]harmine (4.6 MBq/kg body weight). Ninety minutes of PET

200 data were acquired in 3D mode and reconstructed using an iterative filtered backprojection

201 algorithm, which was separated into 51 time frames with a spatial resolution of 4.36 mm with

202 a FWHM of 1 cm next to the center of the field of view and reconstructed in 35 transaxial

203 sectional volumes (128 × 128 matrix). Automatic arterial blood sampling was performed during

204 the first 10 min at a rate of 4 ml/min (ALLOGG, Mariefred, Sweden) and manual samples were

205 taken at 5, 6, 7, 8, 10, 20, 40, 60, and 80 min after bolus start (57). A gamma counter previously

206 cross-calibrated to the PET scanner was used to measure radioactivity concentrations in both

207 whole blood and plasma. Furthermore, samples at 6, 7 and 8 min were used for individual

208 cross-calibration between manual and automated blood sampling. Fractions of radioactive

209 metabolites and the parent compound were determined with HPLC as described previously

210 (58).

MRI data processing

212 Voxel-based morphometric changes were calculated and processed in the CAT12 toolbox

213 (http://www.neuro.uni-jena.de/cat/, version r1742) in MATLAB (version 9.8) using the

214 longitudinal pipeline with default settings unless otherwise specified. Prior to preprocessing,

215 raw data was visually inspected for potential artifacts. In short, the T1 images from both time

216 points were co-registered, resampled and bias-corrected. Each scan was then skull stripped

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217 and segmented into GM, white matter and cerebrospinal fluid, and resulting GMD was further

218 used. Finally, GMD maps were normalized into MNI space and spatially smoothed using an 8-

219 mm Gaussian kernel. Lastly, the CAT12 reports and data were visually controlled for

220 registrations and segmentation errors.

221 As an estimate for GMM changes, DWI data was processed within the FMRIB software library

222 (FSL, v. 6.0.05) (59) and unring (https://bitbucket.org/reisert/unring), ANTs

223 (https://github.com/ANTsX/ANTs), MRtrix (https://www.mrtrix.org/) according to an optimized

224 pipeline (60). All data was brain-masked, corrected for noise, movement, gradient, Gibbs

225 ringing, bias field and eddy current distortions as well as for outliers in the form of signal

226 dropouts (60). The diffusion tensors were fitted using the "dtifit" command as well as the rotated

227 b-vectors and then smoothed using a 1 mm kernel (60). Mean diffusivity (MD) estimates were

228 calculated and co-registered with the participant- and measurement-specific segmented GMD

229 map. Thereafter, all images were normalized into MNI space and subsequently the GMD

230 segmentation was thresholded and used to mask out non-GM voxels. Finally, the DWI MD

231 estimates were smoothed using an 8 mm Gaussian kernel and masked again.

PET data processing and MAO-A quantification

233 MAO-A quantification was carried out using PMOD 3.509 (PMOD Technologies Ltd., Zurich,

234 Switzerland; www.pmod.com). Whole blood data was fitted with the sum of three exponentials

235 from the peak onward, plasma-to-whole blood ratio was fitted with a linear function and the

236 parent fraction was modeled with a Watabe function. The arterial input function (AIF) was

237 obtained by multiplying the fitted whole blood activity, plasma-to-whole blood ratio and the

238 fraction of intact radioligand in plasma. The Logan plot was used to quantify voxel-wise MAO-

239 A total V_T with the thalamus as high uptake region. Regional V_T were extracted using the

240 activation map created from the overlap between GMD and DWI analyses.

Statistics

242 Structural changes determined by the MD DWI metric and GMD were estimated using 4 x 2

243 (group x time) repeated measures ANOVA (rmANOVA) implemented in SPM12. Interactions

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244   between group (TM, TW, CW, CM) by time (before and after min. 4 months of hormone

245   therapy) were examined and further corrected for multiple testing using Gaussian random field

246   theory as implemented in SPM12 and the threshold for significance was set at $p \leq 0.025$ family-

247   wise error (FWE)-corrected according to (61) at the cluster-level following $P \leq 0.001$

248   uncorrected at the voxel-level. Further post-hoc comparisons and number of DWI metrics were

249   adjusted again using the Bonferroni method. To account for the individualized GHT treatment,

250   progesterone, testosterone and estradiol were added to both rmANOVA models as covariates.

251   Furthermore, the total intracranial volume was added as a covariate in the VBM model. For

252   both the GMD and DWI models each covariate was standardized, and group mean centered.

253   To provide a robust identification of hormonally induced gray matter alterations, the different

254   structural changes reflected by both the GMD and DWI analyses were combined. The

255   significant effects from both models were binarized and a conjunction map across both imaging

256   modalities was calculated as an intersection. The remaining brain regions included in this

257   conjunction map were used for the subsequent MAO-A V_T analysis.

258   Interaction effects in MAO-A V_T before and after GHT for both cis- and transgender individuals

259   were estimated using a linear mixed model in SPSS 25.0 (SPSS Inc., Chicago, Illinois;

260   www.spss.com). Here, the global model consisted of group, time and ROI (extracted from the

261   conjunction map) as factors and MAO-A V_T as the dependent variable. The model was reduced

262   in case of non-significance. Paired t-tests were used to estimate changes in SDI scores before

263   and after GHT for both transmales and transfemales ($p_{Bonferroni} < 0.025$). Finally, partial

264   correlations between changes in SDI scores to GMD and GMM while correcting for the main

265   influencing hormone were calculated to investigate a possible link between structural and

266   behavioral changes after GHT ($p_{Bonferroni} < 0.05$). The Bonferroni method was used to correct

267   for multiple comparisons in all tests.

268

269

Results

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271 Demographics and hormone levels are listed in table 1. Fewer TW and matched CM were

272 recruited when compared to TM and CW. Due to irreversible damage to the PET imaging

273 equipment, 35 participants partook in the PET part of the study, where only 17 completed both

274 scans, see table 1 for a detailed breakdown of subgroups. Out of the 35 participants that

275 underwent the PET scans, only 52 scans were suitable for further analysis.

Structural and MAO-A changes after GHT

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277 For both the VBM and DWI analyses, significant 2-way (time-by-group) interaction effects were

278 found. More widespread structural changes after a median of 4.5 months GHT using VBM

279 were discovered, when compared to MD, see figure 1a + b. Combining the results from VBM

280 and DWI analyses showed an overlap of changes in the fusiform gyrus, rolandic operculum,

281 inferior occipital cortex, middle and anterior cingulum and insula, cerebellum and the lingual

282 gyrus (figure 1c). Post-hoc tests revealed changes in GMD, see figure 2 and also for MD, figure

283 3, ($p_{FWE + Bonferroni} < 0.025$). An increase in GMD was found in the fusiform gyrus, cerebellum

284 and lingual gyrus after GHT for transmen compared to both control groups. Transwomen

285 contrastingly showed a decrease in GMD after GHT in the rolandic operculum, insula, posterior

286 cingulum, cerebellum and lingual gyrus when compared to both cismales and cisfemales.

287 The opposite interaction effects were found in the MD of the fusiform and lingual gyrus for

288 transmales compared to both cis control groups. An increase of MD in the insula was also

289 found for transwomen after GHT when compared to both control groups.

290 When investigating regional changes in MAO-A V_T, no interaction and no main effects were

291 significant.

Behavioral changes after GHT

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293 An increase in the overall SDI scale (sum score) "desire with ($t = 5.84$, $p_{Bonferroni} < 0.001$) and

294 without interactions" ($t = 3.4$, $p_{Bonferroni} = 0.01$) was found for TM after GHT. Furthermore, a

295   moderate negative correlation between changes in the SDI sum score of desire with interaction

296   and GMD in the bilateral insula after GHT for the TM group was found (rho = - 0.62, $p_{Bonferroni}$ =

297   0.047). While other regions and sum score of desire without interaction also showed moderate

298   correlations, no others survived the correction for multiple comparisons. Moreover, no

299   significant correlations between GMM and any SDI sum scores were found, p > 0.12.

300

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Discussion

301   The present study aimed to investigate potential changes to GM and MAO-A density induced

303   by GHT over a median time period of 4.5 months in subjects with GD. To reveal robust changes

304   in both GD, morphometric differences in GMD and microstructural alterations as reflects by

305   GMM were combined and subsequently used for assessment of MAO-A V_T. Significant

306   changes in GMD and GMM after GHT that will be discussed in the following section were found

307   in nine distinct brain regions. Across these regions, no significant changes of MAO-A V_T were

308   found. In an additional analysis we probed the effect of GHT on sexual desire as measured by

309   the SDI and found an increase in the SDI sum scores "desire with" and "without interactions"

310   for TM after GHT.

311   **Structural changes after GHT**

312   Significant 2-way (time-by-group) interaction effects induced by GHT, as reflected by both the

313   VBM and DWI analyses, were found in the fusiform gyrus, rolandic operculum, inferior occipital

314   cortex, middle and anterior cingulum, bilateral insula, cerebellum as well as the lingual gyrus.

315   As discussed in the following section, our findings are in line with prior studies reporting

316   significant changes in GMD and GMM. Moreover, in comparison to cross-sectional data (62-

317   66), our longitudinal design allows for more accurate interpretation.

318   *Fusiform gyrus:*

319   As various other regions presenting significant changes to GMD and GMM reported here, the

320   fusiform gyrus is implicated in own-body perception in the context of self (49, 67) and is known

321   to show different functional connectivity patterns in GD when compared to cisgender controls

322   (49). These functionally different patterns might be reflected by structural differences as well.

323   Here, we found a GHT-induced increase of GMD and decrease of MD (as a marker for GMM)

324   in the fusiform gyrus of TM, potentially indicating anticatabolic effects of testosterone (44, 68).

325   *Lingual gyrus, bilateral insula and posterior cingulum:*

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326   After androgenization of TM, Zubiaurre-Elorza and colleagues found a significant increase of

327   cortical thickness in multiple brain regions, e.g., the left lingual gyrus (47). This is in line with

328   our finding, however in the present study, GMD of the lingual gyrus increased and MD

329   decreased bilaterally across TM after high-dose testosterone treatment. Of note, the lingual

330   gyrus is centrally involved in processing visual information and thus visuospatial functioning.

331   As men, on average, perform better than women in visuospatial functioning, and testosterone

332   has been suggested to directly influence visuospatial functioning both in men (69) as well as

333   women (70), it could be hypothesized that advantages in this skill could partially be traced back

334   to an interrelation of higher GMD and and lower and the influence of high-dose testosterone

335   treatment. In addition, we found a significant decrease in GMD across the insula as well as the

336   posterior cingulum of TW when compared to CW and CM. The insula belongs to the salience

337   network (71), which plays a central role for interoceptive processes and own-body perception

338   (67). Differences in functional connectivity were further shown in language and affective

339   processing (72, 73) as well as in various neuropsychiatric conditions (74, 75). However, sex-

340   based differences between cisgender men and women regarding the insula were not only

341   shown in functional MRI, but also in structural analyses (3) and investigations of the GABAergic

342   system in humans (76). Thus, changes to GMD might be seen as a marker for microstructural

343   remodeling induced by estrogen and anti-androgen therapy, as it is needed for multifactorial

344   adaptations that take place throughout GHT (49, 77). Our findings are in line with prior reports

345   on decreased GMD, higher MD reflecting microstructural changes, and widespread cortical

346   thinning in anti-androgen and estrogen treatment across TW (46, 47).

Changes to MAO-A distribution

348   In view of sexually dimorphic characteristics of multiple neuropsychiatric conditions, better

349   understanding of how sex steroids interact with neurotransmitters on a molecular level, e.g.,

350   as reflected by MAO-A density, is of major interest. In the present study, none of the subgroups

351   showed significant changes to MAO-A V_T in any of the brain regions that were found to be

352   different in terms of GMD and GMM after GHT. In contrast, in a prior analysis of MAO-A density

353   after GHT performed by our group, we found statistically significant reductions of MAO-A V_T in

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354   six of twelve previously defined regions of interest in TM after GHT for at least 4 months. These

355   regions included the middle frontal cortex (−10%), anterior cingulate cortex (−9%), medial

356   cingulate cortex (−10.5%), insula (−8%), amygdala (−9%) and hippocampus (−8.5%, all

357   p<0.05)) (33). Of note, in the study by Kranz et al. regional MAOA V_T was estimated based on

358   *a priori* defined regions-of-interest (ROIs) from a modified AAL atlas (33, 78), whereas here,

359   MAO-A analysis was performed in regions that showed significant GMD and GMM changes

360   after GHT.

361   **GHT effects on sexual desire**

362   The effect of GHT on sexual desire found here corroborates the findings of previous observations

363   reporting increased sexual desire in TM after androgen treatment (51, 52, 79-82). Even though

364   Defreyne et al. found that some sexual desire changes observed over the course of GHT might

365   diminish over time, elevated total, dyadic, and solitary SDI scores in TM remained stable on

366   the long-term (52). This is in line with our finding of elevated sexual desire in TM after a median

367   time period of 4.5 months of GHT. Despite the effects quantified 4.5 months after GHT

368   initiation, no further follow-up was performed in the present study. Therefore, a decrease of

369   sexual desire after a longer therapeutic period cannot be ruled out completely. However, a

370   follow-up period of 12 months has been performed by van Dijk and colleagues who reported

371   the most pronounced changes in sexual desire across TM within the first 3 months of treatment

372   which remained largely stable even after 12 months (81, 83). The increase in sexual desire

373   among TM might be traced back to testosterone treatment per se (84) and higher androgen

374   levels in general (85). However, many other factors could have influenced sexual drive, e.g.,

375   more satisfactory sexual interactions and relationships due to transition or improvement in

376   general well-being after GHT (82).

377   Interestingly, partial correlation analyses between changes in SDI scores and GMD/GMM

378   revealed a significant negative correlation between changes in the SDI sum score of desire

379   with interaction and GMD in the bilateral insula of TM individuals. This finding suggests a

380   possible connection between structural GM changes in the insula and changes in sexual desire

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381 after masculinizing GHT. Among many other functions, the insula is essential for the integration

382 of external sensory stimuli via various neuronal pathways (86). Activation of the bilateral insula

383 during sexual arousal and desire has been shown in several fMRI studies (87-89), indicating a

384 link between insular activation and the processing of sexual stimuli. Although there have been

385 multiple fMRI studies in this area, this is, to the best of our knowledge, the first study to link

386 structural GM changes after long-term masculinizing GHT with changes in sexual desire.

387 Moreover, the significance of a negative (rather than positive) correlation between structural

388 GM changes and changes of sexual desire (found in subjects treated with testosterone) has

389 yet to be evaluated. Nevertheless, the finding points to an influence of the hormone on both

390 sexual desire and structural remodeling within cortical regions such as the insula.

391 Of note, also the neurotransmitter dopamine plays a key role in insular activation while

392 processing sexual stimuli (90). Thus, the presence of mesolimbic dopaminergic projections

393 reaching the insula and contributing to the processes taking place during sexual arousal and

394 sexual desire should be considered in future neuroimaging studies in this field (91).

395 **Limitations**

396 Some limitations of the present study need to be addressed. First, subgroups were not

397 sufficiently balanced due to recruitment difficulties, especially in case of subjects with gender

398 dysphoria. Second, the subgroup that underwent PET imaging comprised only 17 individuals

399 due to irreparable damages to the PET equipment. Therefore, it cannot be ruled out that

400 potential GHT-induced changes in MAO-A V_T both on a global as could not be detected in the

401 present analyses.

Conclusion

403 Long-term GHT seems to have a considerable impact on GMD and GMM in the brain of

404 transgender individuals. Specific effects of either androgenizing or feminizing sex steroids

405 must be taken into account in most regions, however, in selected structures both types of GHT

406 affected GMD and GMM in the same way, pointing towards mechanisms that are induced by

407   GHT irrespective of whether feminizing or androgenizing steroids are used. The combined

408   approach in analyzing structural MRI data has the potential to shed light on changes of GM as

409   reflected by both morphometric as well as microstructural analyses after GHT in a longitudinal

410   design and in comparison to cisgender individuals. Nevertheless, larger sample sizes are

411   needed to detect reliable associations between GM and potential MAO-A density changes

412   induced by GHT. In terms of sexual desire, our findings are consistent with the literature and

413   supports evidence from previous observations of increased sexual desire in TM after

414   masculinizing GHT.

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CRediT authorship contribution statement

Patricia A Handschuh; conceptualization, investigation, visualization, writing – original draft, **Murray B Reed**; conceptualization, methodology, software, formal analysis, data curation, visualization, writing – original draft, **Matej Murgaš**; software, data curation, review & editing, **Crysoula Vraka**; review & editing, **Ulrike Kaufmann**; review & editing, investigation, **Lukas Nics**; review & editing, **Marius Ozenil**; review & editing, **Dr. Melisande E. Konadu**; investigation, review & editing, **Manfred Klöbl**; investigation, data curation, writing – review &

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editing, **Eva-Maria Patronas**; review & editing, **Benjamin Spurny-Dworak**; review & editing, **Andreas Hahn**; software, supervision, writing – review & editing, **Marcus Hacker**; review & editing, **Marie Spies**; conceptualization, investigation, writing – review & editing, **Pia Baldinger-Melich**; review & editing, investigation, **Georg S. Kranz**; conceptualization, writing – review & editing, **Rupert Lanzenberger**; project administration, funding acquisition, supervision

Conflict of Interest

With relevance to this work there is no conflict of interest to declare. R. Lanzenberger received travel grants and/or conference speaker honoraria within the last three years from Bruker BioSpin MR, Heel, and support from Siemens Healthcare regarding clinical research using PET/MR. He is a shareholder of the start-up company BM Health GmbH since 2019. G.S. Kranz declares that he received conference speaker honorarium from Roche, AOP Orphan and Pfizer. P. Handschuh received authorship honoraria from MedMedia Verlag and a travel grant from Angelini Pharma. The other authors report no conflict of interest.

Data availability statement

Due to data protection laws processed data is available from the authors upon reasonable request. Please contact rupert.lanzenberger@meduniwien.ac.at with any questions or requests.

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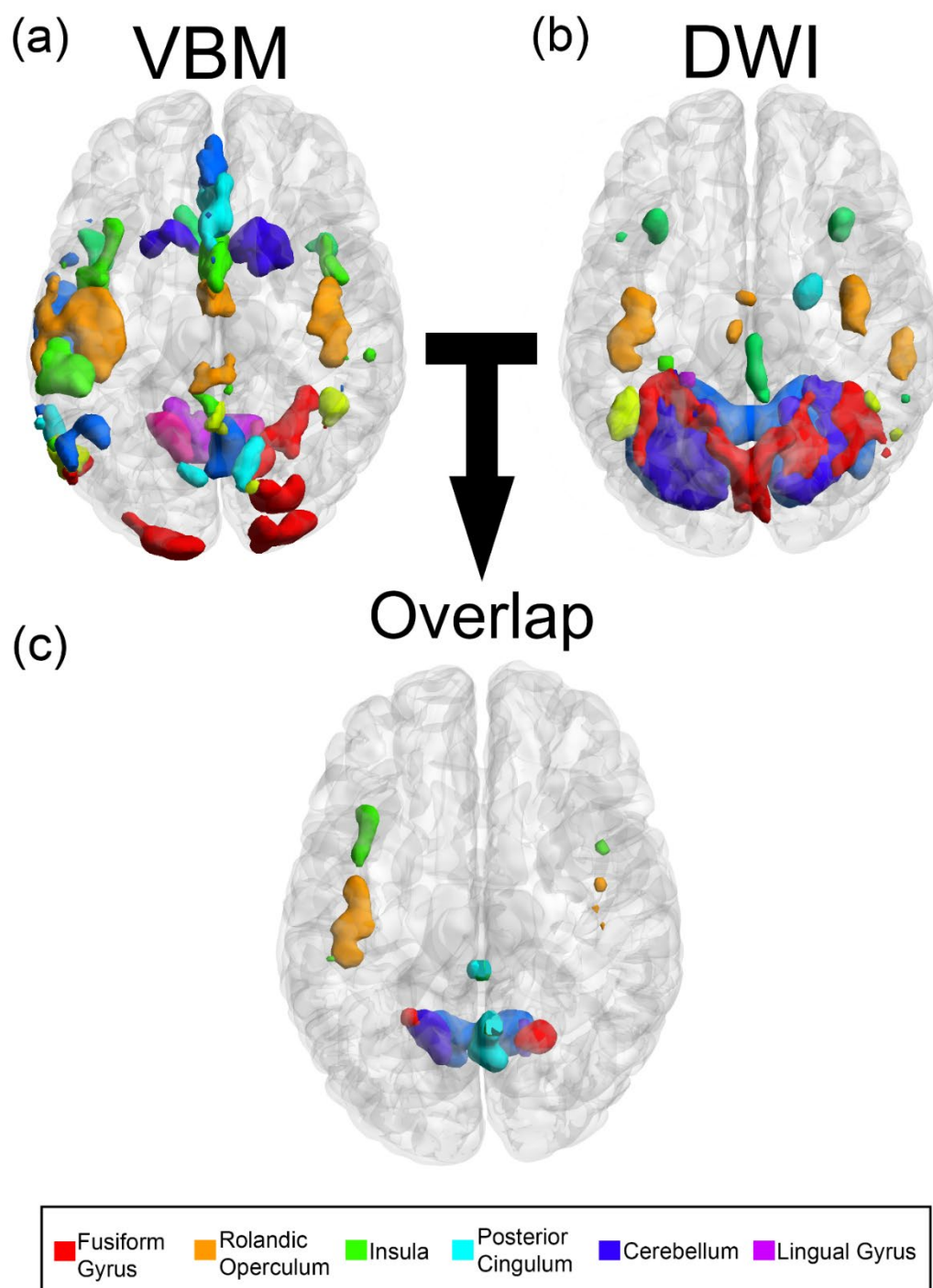718   Behav. 2019;9(12):e01389.

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Figure 1: Structural changes after gender-affirming hormone therapy. *(a) Shows the significant changes derived from the voxel based morphometry (VBM) analysis, whereas (b) shows the mean diffusivity changes in gray matter for all transgender and cisgender groups after a median of 4.5 months of gender-affirming hormone therapy. (c) Indicates the overlapping regions between the VBM and DWI analyses.*
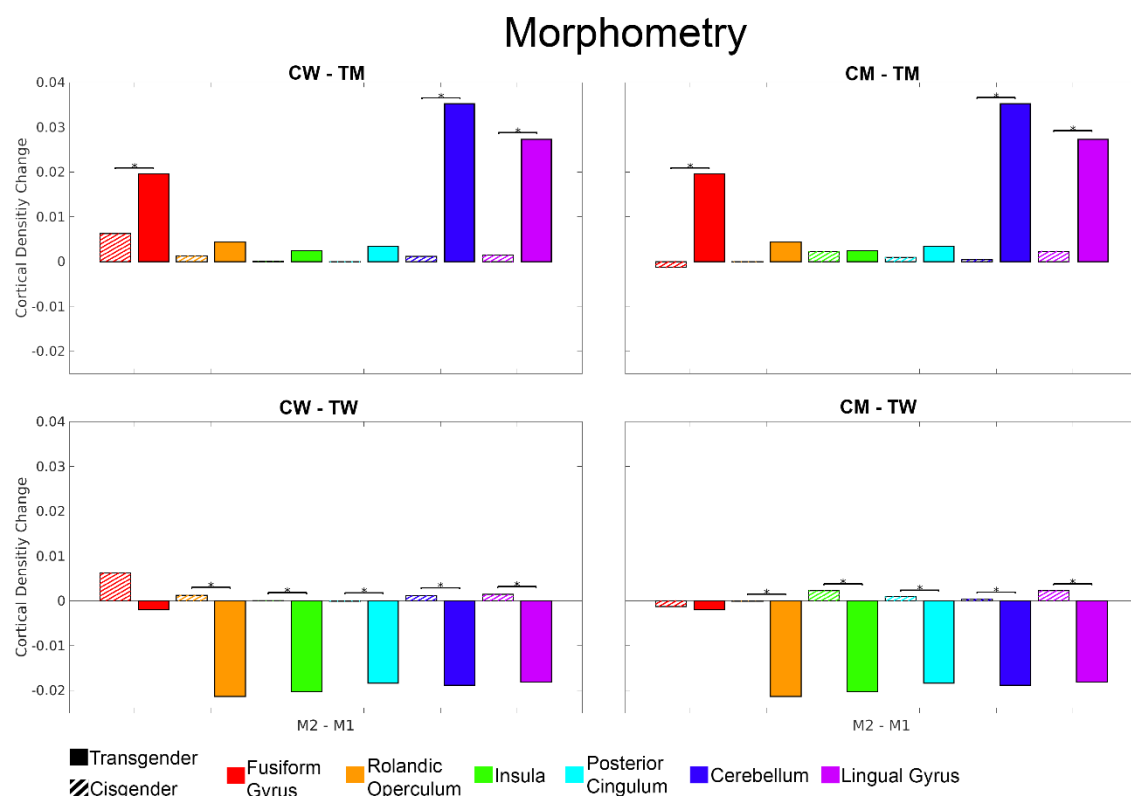
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Figure 2: Voxel based morphometry regional changes. *Each bar represents the change in gray matter density (GMD) after 4 months of gender-affirming hormone therapy (GHT), where the hatched bars represent the cisgender groups of male (CM) and female controls (CW) and the solid bars the transgender groups, transmale (TM) and transfemale (TW). Here widespread regional interactions can be found in the TW group when compared to both CM and CW, whereas TM showed less regional interactions, however the significant interactions showed a greater magnitude of change. An increase in GMD was found TM, whereas a decrease was found in TW after a median of 4.5 months of GHT when compared to both CM and CW. Significant group-by-time interactions are denoted with a *.*
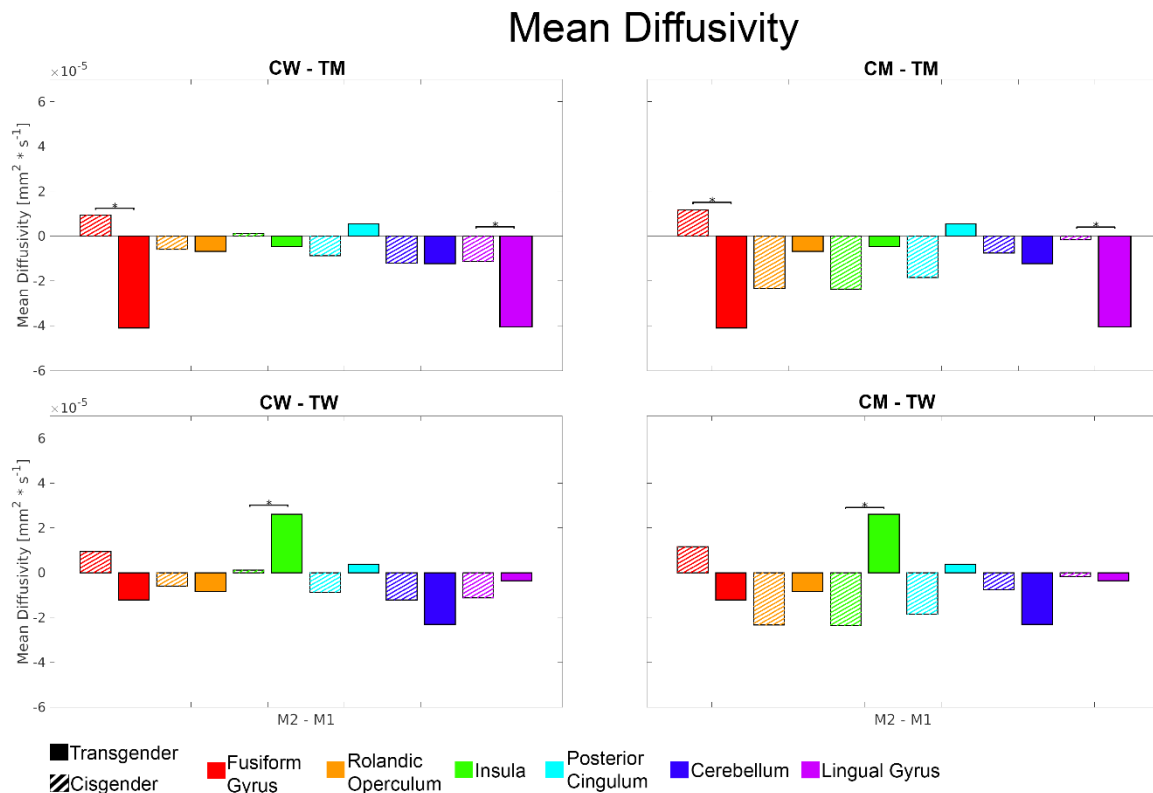
Figure 3: Mean diffusivity regional changes. *Each bar represents the average diffusivity change reflective of microstructural alterations after 4 months of gender-affirming hormone therapy, where the hatched bars represent the cisgender groups of male (CM) and female controls (CW) and the solid bars the transgender groups, transmale (TM) and transfemale (TW). Here, changes a decrease in both TM vs CW or CM and also TW vs CM were found. Significant group-by-time interactions are denoted with a *.*

	cisgender women		cisgender men		transgender women		transgender men	
N(VBM + DWI)	20		11		20		10	
Age [years]	25.3 ± 6.7		26.5 ± 8.0		28.6 ± 9.3		24.75 ± 7.3	
Completed	M1	M2	M1	M2	M1	M2	M1	M2
N(PET)	9	3	8	3	7	5	11	6
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
DHEAS (µg/ml)	3.2 ± 0.9	3.2 ± 1.0	3.8 ± 1.9	2.6 ± 1.3	3.9 ± 1.1	3.8 ± 1.3	3.2 ± 1.9	3.3 ± 1.9
Estradiol (pg/ml)	81.2 ± 56.7	112.1 ± 79.5	22.7 ± 10.3	22.9 ± 9.0	35.7 ± 16.6	290.0 ± 299.1	111.2 ± 74.9	59.3 ± 50.3
FSH (µU/ml)	4.9 ± 1.4	4.9 ± 2.8	3.8 ± 1.6	3.8 ± 1.5	3.3 ± 1.7	0.2 ± 0.1	4.5 ± 2.5	5.6 ± 3.6
LH (µU/ml)	9.8 ± 6.4	9.5 ± 6.9	5.8 ± 1.9	6.5 ± 2.3	6.5 ± 2.9	0.1 ± 0.1	9.6 ± 5.9	8.6 ± 8.8
Progesterone (ng/ml)	1.3 ± 1.3	4.9 ± 5.6	0.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	0.8 ± 0.9	4.9 ± 6.4	0.3 ± 0.2
SHBG (nmol/L)	67.4 ± 28.5	69.6 ± 27.6	45.3 ± 25.0	43.2 ± 19.3	45.7 ± 21.5	70.2 ± 39.4	65.3 ± 36.3	36.3 ± 15.1
Testosterone (ng/ml)	0.4 ± 0.2	0.3 ± 0.1	5.6 ± 2.1	5.0 ± 1.2	5.7 ± 1.7	0.3 ± 0.2	0.4 ± 0.2	4.3 ± 2.1

750 **_Table 1: Demographics of all participants for both DWI + VBM and PET analyses_**_, each_

751 _divided into their respective subgroups. Age is given from the inclusion date. Hormone levels_

752 _for the pre-treatment assessment and cisgender participants are provided for comparison only_

753 _and were not used as covariates in the statistical analyses._

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