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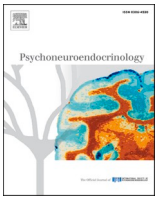

The influence of sex steroid treatment on insular connectivity in gender dysphoria

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ABSTRACT

Background: Sex-specific differences in brain connectivity were found in various neuroimaging studies, though little is known about sex steroid effects on insular functioning. Based on well-characterized sex differences in emotion regulation, interoception and higher-level cognition, gender-dysphoric individuals receiving gender-affirming hormone therapy represent an interesting cohort to investigate how sex hormones might influence insular connectivity and related brain functions.

Methods: To analyze the potential effect of sex steroids on insular connectivity at rest, 11 transgender women, 14 transgender men, 20 cisgender women, and 11 cisgender men were recruited. All participants underwent two magnetic resonance imaging sessions involving resting-state acquisitions separated by a median time period of 4.5 months and also completed the Bermond-Vorst alexithymia questionnaire at the initial and final examination. Between scans, transgender subjects received gender-affirming hormone therapy.

Results: A seed based functional connectivity analysis revealed a significant 2-way interaction effect of group-by-time between right insula, cingulum, left middle frontal gyrus and left angular gyrus. Post-hoc tests demonstrated an increase in connectivity for transgender women when compared to cisgender men. Furthermore, spectral dynamic causal modelling showed reduced effective connectivity from the posterior cingulum and left angular gyrus to the left middle frontal gyrus as well as from the right insula to the left middle frontal gyrus. Alexithymia changes were found after gender-affirming hormone therapy for transgender women in both fantasizing and identifying.

Conclusion: These findings suggest a considerable influence of estrogen administration and androgen suppression on brain networks implicated in interoception, own-body perception and higher-level cognition.

1. Introduction

The insula is located within the lateral sulcus of both hemispheres of the brain, covered by the frontoparietal and the temporal opercula. Its broad structural connectivity to multiple brain regions is reflective of its wide range of functions. Highly dynamic processes involving the interaction of emotion and cognition have been linked to the insula, encompassing the regulation of explicit emotional experiences and the integration of somatosensory as well as autonomic stimuli in the context of cognitive processing (Namkung et al., 2017). Analyses on coactivation patterns of the insular cortex suggest the primary involvement of the ventral anterior part in affective regulation, whereas the dorsal anterior part is rather implicated in cognitive processing (Chang et al., 2013). Sensorimotor activation and interoceptive functions are largely attributed to the posterior insula (Craig, 2002). However, this tripartite parcellation is called into question by functional heterogeneity within certain subdivisions as well as the dense interconnection across them (Uddin et al., 2014). There is also evidence of differences between the function of the right and left insula; specifically, the right insula has been shown to play an important role in

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interoceptive awareness and emotional salience (Critchley et al., 2004; Eckert et al., 2009). In line with these findings, Scalabrini et al. found higher context-dependent functional connectivity between the right insula and other regions implicated in self-related processing when compared to the left insula. Furthermore, the authors conclude that the neural activity of the right insula differs from the left insula and other self-related regions. In this study, functional magnetic resonance imaging (fMRI) was used (Scalabrini et al., 2021).

Next to structural alterations (Goodkind et al., 2015), altered functionality of the insular cortex was shown in major depression, bipolar disorder, anxiety disorders or schizophrenia (Janiri et al., 2020; Moran et al., 2013; Yin et al., 2018) as well as in neurologic conditions, e.g., Parkinson's disease and various forms of dementia (Fathy et al., 2020). Given that the insula is known to be a central region for interoception, emotional awareness and in general for the integration of internal feelings as well as external emotional stimuli, it seems plausible that many neuroimaging studies found an association between reduced insular activation (Bird et al., 2010; Silani et al., 2008) and alexithymia as well as between insular damage and acquired alexithymia (Hogeveen et al., 2016; Williams and Wood, 2010). Of note, alexithymia refers to a subclinical condition expressing the reduced ability to identify and accurately describe emotions or a reduced awareness for emotional states (Taylor, 2000).

Significant sex differences in alexithymia are well documented. In a meta-analysis performed by Levant et al., men were found to show higher levels of alexithymia, irrespective of their clinical status (Levant et al., 2009). Interestingly, alexithymia seems to be more frequent in trans- than in cisgender individuals. In a recently published study by Kallitsounaki and Williams, nonautistic transgender participants were found to show significantly higher mean levels of alexithymia than nonautistic cisgender participants (Kallitsounaki and Williams, 2023). Similar findings were published by Mazzoli et al., who also found significantly higher levels of alexithymia in transgender individuals. Additionally, they reported a significant decrease in alexithymia after gender-affirming hormone therapy (GHT) (Mazzoli et al., 2022).

Considering the brain as an organ with sex-specific[3] differences at every age, the influence of sex hormones (Toffoletto et al., 2014; Witte et al., 2010) and sex in general have been addressed in various neuroimaging studies, highlighting typical divergences between healthy male and female brain functioning. In language processing, female subjects were found to show greater activation within the right insula compared to their male counterparts (Harrington and Farias, 2008). Regarding affective processing, Duerden et al. found that emotional stimuli mainly activated the left anterior, mid as well as right posterior insula in men. In contrast, the bilateral anterior insula and the left mid and posterior insula were activated in female subjects (Duerden et al., 2013). Additionally, in a quantitative meta-analysis of neuroimaging studies on sex differences in brain activation in response to negative and positive emotional stimuli, Stevens and Hamann found that in women, an activation of the left insula could be observed after negative emotional stimuli (Stevens and Hamann, 2012). In men, however, bilateral insular activation was found in response to the same stimuli (Duerden et al., 2013). In terms of interoception, Galli and coworkers found strong evidence for sex-related differences in the activation of the insula following the termination of an aversive interoceptive stimulus (Galli et al., 2013).

As reviewed by McEwen and colleagues, sex hormones are known to be involved in neurotransmitter metabolism and neuroplasticity in many ways (McEwen and Alves, 1999; McEwen and Milner, 2017). Sex steroid hormones exert their effects via membrane associated cell surface and intracellular receptors. In particular, estrogen responsive receptors are found in various regions and cell types throughout the brain, e.g., in the hippocampus (Daniel and Dohanich, 2001; Murphy et al., 1998; Packard et al., 1996), in the prefrontal cortex (PFC) and the amygdala (Shansky et al., 2010; Zeidan et al., 2011) as well as in the cerebellum, e.g., affecting spine and synapse formation (Sakamoto et al., 2003) as well as glutamatergic neurotransmission via local cerebellar estradiol synthesis (Azcoitia et al., 2011). In dopaminergic cells, estrogen is known to exert its effects mainly via membrane-associated estrogen receptors across the PFC, dorsal striatum, nucleus accumbens, and hippocampus (Almey et al., 2015). In most species, the amount of estrogen receptors (ERα and ERβ) within the insula and the PFC is only moderate when compared to other brain regions (Shughrue et al., 1997). Nevertheless, direct effects of sex steroid hormone manipulation on insular function have been suggested, which may be explained by the important role of the insular cortex as a connective hub on the one hand and by the wide range of sex-specific differences in many brain functions affected by the insula on the other. For instance, Macoveanu et al. found a link between changes in testosterone levels after the administration of GnRH agonists to healthy cycling women and changes in bilateral insular activation in response to monetary rewards (Macoveanu et al., 2016). In a similar fMRI-study with GnRH agonists, Henningsson et al. could demonstrate an association between GnRH agonist-induced mood changes and emotion-elicited activation of the anterior insula (Henningsson et al., 2015). Testosterone, in general, seems to play a pivotal role in especially male depression. It is known to be a key agent for the regulation of the HPG axis via kisspeptin signaling. Additionally, testosterone seems to regulate kisspeptin neurons in the limbic system (e.g., amygdala and hippocampus) (Hauger et al., 2022). Looking at the insular cortex, Banczerowski and coworkers found that in male rats, damage to the right insula resulted in a significant reduction in basal testosterone secretion, indicating an essential role of the right insula for the regulation of testosterone and LH secretion (Banczerowski et al., 2001). Another rat study published by Kritzer and Creutz revealed sex-specific specializations in androgen receptor localization that "paralleled established patterns of mesocortical hormone sensitivity, including the androgen sensitivity of dopamine axons and dopamine-dependent functions in prefrontal cortex" (Mary and Lela, 2008). While little is known about how testosterone interacts with insular cortex activation, Burke et al. has shown changes to cortical thickness after testosterone treatment in transgender (TX) men (TM) (Burke et al., 2017).

Sex-specific changes of the insula were also shown in individuals suffering from neuropsychiatric conditions such as major depressive disorder (Eisenberger et al., 2009), chronic pain (Gupta et al., 2017), schizophrenia (Mikolas et al., 2016) or migraine (Maleki et al., 2012), just to name a few.

A unique possibility to further investigate the specific, direct effects of sex hormones on brain functionality is given by cohorts diagnosed with gender dysphoria (GD) undergoing GHT. As concisely reviewed by Kranz et al., GHT was shown to modulate brain structure and function in numerous ways (Kranz et al., 2020). Although little is known about the effects of GHT specifically on the insular cortex, previous neuroimaging studies that have examined the transgender brain either using a whole-brain-approach or focusing on specific networks or regions of interest have found some highly interesting effects of hormonal interventions on, among other regions, the insula. On a molecular level, Kranz et al. found a statistically significant reduction of insular MAO-A distribution volume in TM receiving exogenous testosterone (Kranz et al., 2021), probably contributing to functional changes driven by altered neurotransmitter metabolism. In a study by Hahn et al., significant differences of structural connectivity in local efficiency were found between cis- and transgender cohorts, for instance in the right insula of

[3] Sex refers to the biological sex assignment at birth and is usually based on a set of biological characteristics. Gender, on the other hand, refers to socially and culturally constructed roles based on so-called feminine and masculine characteristics, attributes and behaviors that a given culture typically associates with a person's sex assigned at birth (cf. APA, 2015. Guidelines for psychological practice with transgender and gender nonconforming people. American psychologist 70, 832–864.).

TM individuals (Hahn et al., 2015).

Some fMRI studies reported on sex-specific differences of insular connectivity in which, however, varying study designs were used and comparability is therefore limited. Smith et al. found that in cisgender men (CM), insular cortex activation in response to female voices was reduced, while the activation patterns of TM and cisgender women (CW) were characterized by little or no differentiation between male and female voices, pointing towards an activation pattern more in line with the gender identity rather than the sex assigned at birth (Smith et al., 2018). In a study by Uribe et al., connectivity in seeds within the insula and the cingulate cortex also resulted to be weaker in TM than in CM (Uribe et al., 2020). Similarly, Manzouri and coworkers found that TM had significantly weaker resting-state (rs) connections between various brain regions, e.g., the insula when compared to CM and CW (Manzouri et al., 2015). Furthermore, Nawata et al. found a significantly higher regional cerebral blood flow in the right insula of transgender individuals with gender dysphoria when compared to cisgender controls (Nawata et al., 2010).

Considering the importance of the insula as a central anatomical and functional integration hub between many larger cortical and subcortical brain regions involved in cognitive, emotional and sensory processing found to be influenced by sex hormones, the present study aimed to investigate the potential influence of long-term GHT administration on insular connectivity. We included four study groups encompassing TX women (TW) and TM as well as two control groups comprising CW and CM to provide a thorough overview of sex-specific differences in and hormonal effects on insular connectivity. We hypothesize that baseline differences in insular connectivity between TM, TW, CM and CW will be found and that GHT will have a significant effect on insular connectivity with TX individuals showing changes of insular connectivity patterns. We further hypothesize that changes of alexithymic symptoms after GHT will correlate with changes of insular connectivity and that alexithymic symptoms will decrease after GHT.

2. Methods

This study was conducted according to the Declaration of Helsinki including all current revisions and the good scientific practice guidelines of the Medical University of Vienna. The protocol was approved by the institutional review board (EC number 1104/2015) and registered at clinicaltrials.gov (NCT02715232).

2.1. Study Design

The study was conducted in a longitudinal design. All participants underwent two MRI sessions separated by a median of 4.5 months. Each session included structural, diffusion-weighted, task-based and rs functional MRI as well as spectroscopy, but herein only structural and rsfMRI was used. During the time between the two MRI sessions, TX subjects received an individualized GHT treatment regimen that comprised the administration of testosterone and progestins (if menstruation still occurred) in TM, whereas TW received a steroidal antiandrogen and estradiol. Hormone levels were determined by blood draw at each MRI session.

During the preliminary and final examination, participants were required to complete the Bermond–Vorst Alexithymia Questionnaire (BVAQ) (Vorst and Bermond, 2001) to help assess their emotional awareness, social attachment, and interpersonal relations. The presence of depression and depression severity were also assessed by a trained clinician before and after GHT via the Hamilton Depression Rating Scale (Hamilton, 1960) with 24 items (HAM-D24) (Bech, 2015).

2.2. Gender-affirming hormone therapy

Hormone therapy was conducted according to the following protocol implemented at the Department of Obstetrics & Gynecology, Division of Gynecologic Endocrinology and Reproductive Medicine, Medical University of Vienna, Austria: TM received either 1000 mg testosterone undecanoat every 8–16 weeks (Nebido 1000 mg/4 ml i.m.), or alternatively 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 still occurred, TM were treated with either lynestrenol (Orgametril 2–3 tablets/day) or desogestrel daily (Moniq Gynial or Cerazette 0.075 mg 1 tablet/day). 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) or oral estradiol (Estrofem 2x2mg/day p.o.). Additionally, 2.5 mg of an alpha-5-reductase-inhibitor was administered every second day (Finasterid Actavis/Arcana/Aurobindo) in case of extensive hair loss. In selected cases, subjects received GnRH-analogues like triptorelin (decapeptyl 0.1 mg/day subcutaneously).

2.3. Sex hormones

For monitoring sex hormone levels, luteinizing hormone, follicle-stimulating hormone, progesterone, estradiol, testosterone, sex hormone binding globulin and dehydroepiandrosterone sulfate were determined from blood drawn before each MRI session using the quantitative electrochemiluminescence immunoassay method (ECLIA) at the Department of Laboratory Medicine, Medical University of Vienna, Austria (http://www.kimcl.at). Testosterone, progesterone, and estradiol were used as covariates in the analyses. See Table 1 for the exact values.

2.4. Participants

TX individuals seeking GHT were recruited from the Unit for Gender Identity Disorder at the General Hospital in Vienna. Control subjects were recruited via social media, designated message boards at the Medical University of Vienna and at other universities in Vienna. CM and CW were age and education level matched to TM and TW, respectively. Inclusion criteria comprised a diagnosis of gender dysphoria according to the Diagnostic and Statistical Manual for Mental Disorders (TX participants only), version 5 (DSM-5: 302.85) or the International Classification of Diseases, version 10 (ICD-10: F64.1); as well as general health based on medical history, physical examination, electrocardiogram, laboratory screening and structural clinical interview (SCID) for DSM-V Axis I. All participants gave written informed consent to partake in this study, were insured and received reimbursement for their participation. Participants were excluded in case of major neurological or internal illnesses, pregnancy, any kind of psychiatric diagnosis (cisgender controls) or DSM-IV Axis-I comorbidities (TX subjects), steroid hormone treatment as well as treatment with hormonal contraceptives within 6 months prior to inclusion, treatment with psychotropic agents, clinically relevant abnormal laboratory values, MRI contraindications, current substance abuse (excluding smoking), hormonal contraception, current or past substance-related disorder or non-compliance. Of note, TX subjects fulfilling the diagnostic criteria of major depressive disorder or any other type of psychiatric disease were not included to the study. For more demographic information (e.g., sexual orientation or status of education), see supplementary figure 1 and 2.

2.5. Data acquisition

All MRI data were recorded on a Siemens Prisma 3 T scanner using a 64-channel head coil. A whole-brain T1-weighted scan TE / TR = 2.91 / 2000 ms; inversion time = 900 ms; flip angle = 9°; matrix = 240 × 256, 176 slices; 1.0 mm^3; acquisition time = 7:59 min

The rs data was acquired using the following parameters, TE / TR = 30 / 2050 ms; GRAPPA 2; 210 × 210 mm field of view, 100 × 100 pixel in-plane resolution; 35 axial slices of 2.8 mm (25% gap); flip angle 90°;

Table 1
Demographics of all participants and divided into their subgroups. Age is given from the 1st measurement. Hormone levels for the pre-treatment assessment are provided for comparison only and were not used in the statistical analyses. Transwomen showed increases in estradiol and SHBG post-treatment. While, transmen showed increases in testosterone and decreases in estradiol, progesterone and SHBG after gender-affirming hormone therapy.

	cisgender women		cisgender men		transgender women		transgender men	
N	20		11		11		19	
Age [years]	25.09 ± 6.33		27.40 ± 7.22		27.15 ± 13.54		24.95 ± 7.55	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
DHEAS (µg/ml)	3.04 ± 0.95	3.06 ± 1.05	3.99 ± 1.72	4.03 ± 1.38	3.87 ± 1.16	3.64 ± 1.39	3.17 ± 2.01	3.32 ± 1.94
Estradiol (pg/ml)	79.76 ± 54.59	105.86 ± 74.12	25.63 ± 10.21	22.63 ± 8.16	45.70 ± 47.66	271.09 ± 309.90	106.34 ± 73.62	59.57 ± 51.72
FSH (µU/ml)	4.95 ± 1.32	4.74 ± 2.56	3.77 ± 1.59	3.96 ± 1.52	3.87 ± 2.20	0.94 ± 2.93	4.34 ± 2.47	5.29 ± 3.46
LH (µU/ml)	9.58 ± 6.27	8.57 ± 6.72	5.59 ± 1.76	6.68 ± 2.34	7.18 ± 4.70	2.36 ± 8.34	8.99 ± 5.46	7.45 ± 7.20
Progesterone (ng/ml)	1.25 ± 1.29	4.33 ± 5.25	0.40 ± 0.26	0.34 ± 0.23	0.30 ± 0.20	0.83 ± 0.81	5.15 ± 6.50	0.31 ± 0.16
SHBG (nmol/L)	70.78 ± 29.03	71.93 ± 26.24	40.02 ± 23.44	39.41 ± 18.34	42.76 ± 20.46	68.06 ± 52.54	66.54 ± 36.87	36.60 ± 15.45
Testosterone (ng/ml)	0.39 ± 0.17	0.33 ± 0.13	5.36 ± 1.99	5.07 ± 1.25	5.20 ± 2.25	0.48 ± 0.69	0.43 ± 0.20	4.39 ± 2.11

orientation parallel to the anterior-posterior commissure line, with a total acquisition time of 8:09 min. During rs acquisition, participants were instructed to keep their eyes open and let their minds wonder while focusing on a fixation cross projected on a screen within the scanner. The latter was done to reduce head movement.

2.6. fMRI preprocessing

Each participant's data was preprocessed using the following steps: First, physiological artifacts were reduced using PESTICA (Beall and Lowe, 2007). Further preprocessing steps were performed using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) using standard parameters as follows: Slice-timing correction was performed to the temporal middle slice, followed by a two-pass realignment of both measurements per subject to the mean image. The images were then normalized to the Montreal Neurological Institute (MNI) standard space and resliced to 2.5 mm^3. The BrainWavelet Toolbox (Patel et al., 2014) was used to reduce nonlinear artifacts where "chsearch" was set to "harsh" for an increased artifact recognition and "threshold" was set to "20". The images were gray-matter masked with a custom template and finally smoothed using a Gaussian kernel of three times the resliced voxel size. Further processing steps included a Friston-24 model of motion correction (Friston et al., 1996) and an adapted compCor approach using an automatically derived number of combined white matter and cerebrospinal fluid regressors (Behzadi et al., 2007; Klöbl et al., 2020).

2.7. Structural data preprocessing and seed extraction

In parallel, anatomical scans of all participants were preprocessed using the longitudinal recon-all stream (Reuter et al., 2012) and segmented using the Desikan-Killiany atlas (Desikan et al., 2006) in subject space via FreeSurfer 6. Finally, the right insula of each subject was identified and each voxel's time course was extracted within the mask from each individual's preprocessed RS scans in subject space. The extracted time courses were then corrected for all confounders (despiking, bandpass filter: 0.01–0.1 Hz, CompCor) and finally summarized to its first eigenvariate to match the preprocessing pipeline of the RS data.

2.8. Seed Based Connectivity

Seed based connectivity (SBC) was calculated using the right insula's time course previously extracted from the RS data. The seeds time course was correlated with each of the RS pipeline processed voxels' time course. The resulting individual correlation maps were Fisher z-transformed and entered into a repeated-measures ANOVA model using SPM12. Interactions of group (CM, CW, TM, TW) by time (before and after min. 4 months of hormone therapy) were examined.

To investigate potential regional effects within the insula, K-means clustering was used to differentiate the seed region by grouping dynamically similar voxels together. The Bayesian Information Criteria was used to select the optimal number of clusters or sub regions. This was repeated 10 times to attain a robust estimation.

2.9. Effective Connectivity

To determine the directionality of the SBC analysis in the groups that showed a significant interaction, effective connectivity was assessed using spectral dynamic casual modelling (DCM) implemented in SPM12, where the first temporal eigenvariates of clusters from the SBC analyses surviving multiplicity correction were extracted from the data preprocessed for the DCM analysis (Klöbl et al., 2022; Reed et al., 2022). Next, fully connected linear spectral two-state DCMs were estimated (Zeidman et al., 2019a), meaning no constraints were set on any on the nodes or connections. The parametric empirical Bayes (PEB) framework in SPM12 was used for group inference. A flat PEB model (non-hierarchical PEB) was employed and used to analyze changes in free energy (Zeidman et al., 2019b) before and after GHT. Bayesian model averaging (Friston et al., 2016) was utilized to prune connections with high uncertainty posterior probability > 0.99.

2.10. Global Functional Connectivity

Global functional connectivity (GFC) was determined for each participant using the preprocessed data. For each voxel in the grey matter, the GFC was estimated by computing seed-based Pearson correlations of the BOLD signal between the seed and every other grey matter voxel within the brain. The maps where then Fisher z-transformed and averaged across all grey matter voxels. The resulting 3D GFC coefficient map for each participant and measurement was then entered into a repeated measures ANOVA in SPM12, corresponding to the SBC analysis.

2.11. Statistical Analysis

Second-level analyses for SBC and GFC were corrected for multiple testing using Gaussian random field theory as implemented in SPM12 and the threshold for significance was set at $P < 0.025$ family-wise error (FWE)-corrected according to (Chen et al., 2019) at the cluster-level following $P < 0.001$ uncorrected at the voxel-level. All post-hoc comparisons were adjusted multiplicity using the Sidak method.

Progesterone, testosterone and estradiol were used as group level covariates in both the repeated-measures ANOVA model and DCM, to account for the individualized GHT. For both the SBC, GFC and DCM analyses, each covariate was standardized, and group mean centered before being added to the above-mentioned models. Menstrual cycle timing was not included as a covariate as this data was not collected during the study.

In an explorative analysis, mean connectivity metrics were extracted

from regions displaying a significant interaction and then correlated with their change in each of the five BVAQ factors separately, namely analyzing, emotionalizing, fantasizing, identifying, verbalizing. Furthermore, paired t-tests were used to estimate the changes in alexithymia and depression scores before and after GHT ($p < 0.05$) for groups showing a significant interaction in the SBC analysis. Due to the exploratory nature, no correction for multiple comparisons was applied.

3. Results

Demographics and hormone levels are provided in Table 1. Fewer TW and matched CM were recruited when compared to TM and CW (see Table 1). The Bayesian information criterion revealed an optimal solution of 2 sub regions when analyzing the right insular time course using k-means clustering. After visual inspection of the location of both clusters it was found that the 1st cluster represented the insula as a whole. Whereas, the 2nd cluster only outlined the insula, representing a noise component. Therefore, as the right insula time course was considered homogeneous and no further sub region analyses was performed.

3.1. Effective and seed-based network connectivity effects

For the SBC analysis, a significant 2-way interaction of group-by-time was discovered. Post-hoc test revealed that the right insula (seed region) showed increased connectivity with the posterior cingulum (PC; $T = 4.69$; $p_{corr} = 0.047$), left middle frontal gyrus (MFG; $T = 4.60$; $p_{corr} = 0.037$) and left angular gyrus (AG; $T = 4.38$; $p_{corr} = 0.028$) after GHT in the TW group when compared to CM (Fig. 1), but not with any other group. Furthermore, no other significant group-by-time interactions were found. When performing the analysis without covariates in a separate analysis, no interaction effects were found ($p > 0.05$).

To further assess the directionality of the significant SBC interaction, effective connectivity for these groups was estimated. GHT-by-time interactions in effective connectivity for TW participants compared to CM revealed an increase from the right insula to the left AG and left MFG to the PC after GHT. Further, decreases were discovered from the PC and left AG respectively to the left MFG after GHT. Finally, a very slight decrease in effective connectivity from the right insula to the left MFG was revealed after GHT; see Fig. 2 for a graphical representation.

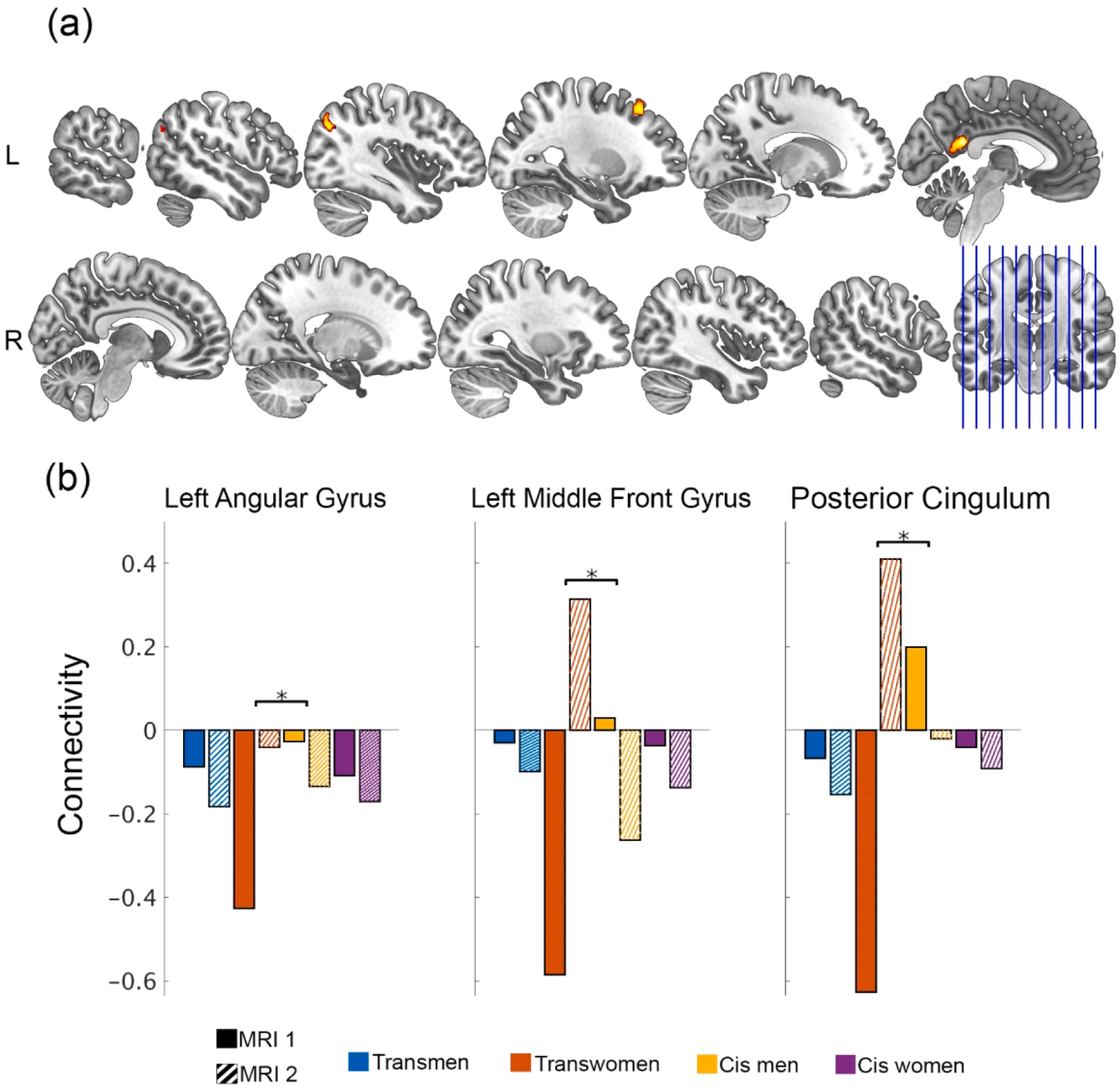

Fig. 1. Seed-based Connectivity. (a) Regions that showed a significant group-by-time interaction with the seed region of the right insula. After a minimum of 4 months an increase in connectivity was found in the posterior cingulum ($p_{corr} = 0.047$ $T = 4.65$), left middle frontal gyrus ($p_{corr} = 0.037$ | $T = 4.60$) and left angular gyrus ($p_{corr} = 0.028$ | $T = 4.38$) for transgender women in comparison to cisgender men. (b) indicates the median connectivity extracted from each region of interest for each MRI session and seperated into the four examined groups. A star [*] indicates a significant interaction of group-by-time.

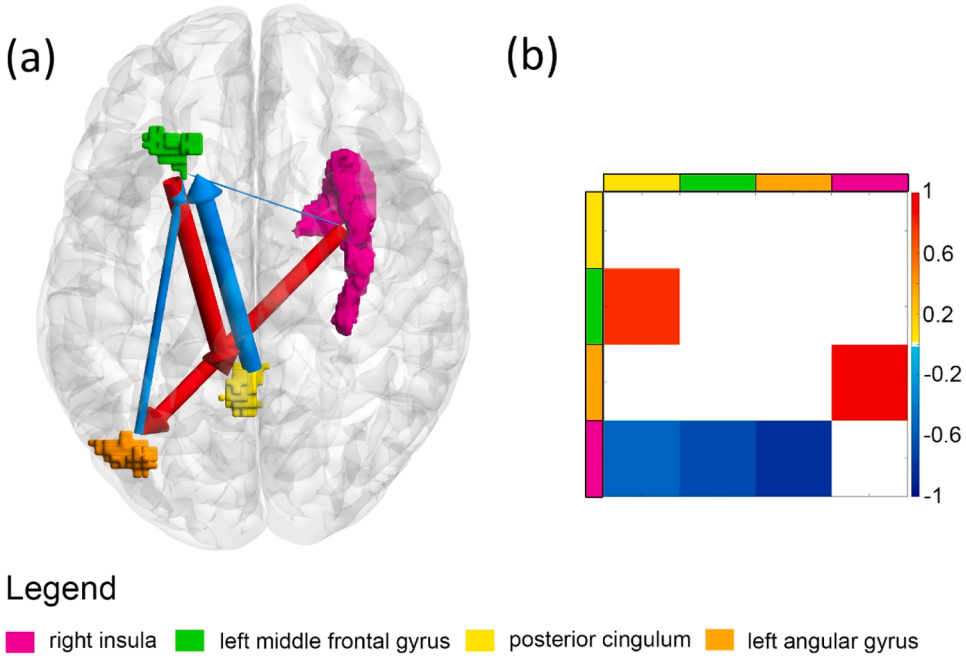

Fig. 2. Effective connectivity. (a) visualizes the directional effective connectivity for the significant group-by-time interaction extracted from the seed based connectivity analysis. This shows an increased effectivity connectivity from the right insula (seed) to the left angular gyrus. A further increase in effective connectivity can be seen from the left middle frontal gyrus to the posterior cingulum. Decreases in connectivity were found from the left angular gyrus and posterior cingulum to the left middle frontal gyrus. A final decrease was found from the left middle frontal gyrus to the right insula. (b) shows the magnitute of the effective connectivity interaction between transwomen and cis men, before and after a minimum of 4 months of gender affirming hormone theraphy. All effects are in relation to the reference conditions, i.e., after a minimum of 4 months of hormone therapy, where transgender women were compared to cisgender men (reference). Increases in effective connectivity are indicated in red and decreases in blue. Line thickness represents the strength of the effective connectivity difference. All effects have a > 0.99 posterior probability.

3.2. Global functional connectivity

The GFC analysis also revealed a significant 2-way group-by-time interaction comprising of the right insula ($p_{ucorr} = 0.023$), right inferior frontal gyrus ($p_{uncorr} = 0.012$) and left inferior temporal gyrus ($p_{uncorr} = 0.02$). After correction for multiple comparisons, none of the above regions survived.

3.3. Alexithymia changes after GHT

The correlation analysis between connectivity measures and each BVAQ factor revealed no significant relationships but did show a trend between the connectivity change in the PC and the identifying factor for TW ($\rho = 0.41$, $p_{uncorr} = 0.089$). The paired t-tests indicated an increase in the identifying factor for TW ($t = 2.14$, $p = 0.04$) and a decrease in the fantasizing factor ($t = -2.29$, $p = 0.03$) after GHT. All other factors in the BVAQ showed no significant change ($p > 0.15$). No longitudinal changes in HAM-D24 scores were found for both cisgender and TX groups before and after GHT ($p > 0.3$). Table 2 displays for a detailed overview of the BVAQ sub scores for each group.

4. Discussion

The current study aimed to assess changes to insular connectivity at rest under gender-affirming hormone treatment. In a seed-based analysis we found that in comparison to CM, TW showed an increase in effective connectivity from the right insula to the left AG and from the left MFG to the PC at rest after receiving GHT. On the other hand, significantly decreased effective connectivity could be shown both from the PC as well as the left AG to the left MFG and from the right insula to the left MFG in the same group comparison. Furthermore, in TW we found an increase in the identifying factor and a decrease in the fantasizing factor of the BVAQ after GHT. No longitudinal changes in HAM-D24 scores were found after GHT in any of the groups.

In this study, long-term GHT resulted in connectivity changes in regions implicated in numerous processes, such as interoception, own-body perception, affective processing and higher-level cognition. As reviewed by Berntson et al., interoception is a highly complex central nervous function involving the identification, processing and integration of signals via different neuronal pathways, networks and circuits (Berntson and Khalsa, 2021). For the integration of sensory input to orchestrate behavior and attention, both the central autonomic network (CAN) as well as the salience network (SN) are needed (Beissner et al.,

Table 2
Mean and standard deviation of Hamilton depression rating scale HAM-D24) scores and Bermond–Vorst Alexithymia Questionnaire (BVAQ) subscores of all participants divided into their subgroups. Moreover, mean and standard deviation of the time, in days, between scan 1 and 2 are divided into subgroups. Although some transgender patients met the criterion for mild depression according to the HAM-D score, they did not meet the general clinical criteria for a depressive episode.

			Cisgender women	Cisgender men	Transgender women	Transgender men
M1	**HAM-D24**	**Sum**	0.7 ± 1.1	0.6 ± 0.8	5.4 ± 6.6	2.5 ± 2.9
	BVAQ	**Analyzing**	8.1 ± 2.2	9.1 ± 1.9	8.9 ± 2.1	10.1 ± 2.5
		Emotionalizing	10.5 ± 2.4	13.4 ± 3.2	10.5 ± 2.6	12 ± 2.3
		Fantasizing	11.3 ± 4.3	13.1 ± 3.7	8.8 ± 2.5	10.8 ± 3.5
		Identifying	7.5 ± 2.2	7.4 ± 1.8	7.6 ± 1.7	8.3 ± 2.6
		Verbalizing	8.5 ± 2.6	10.7 ± 3.7	9.0 ± 3.4	11.5 ± 3.8
M2	**HAM-D24**	**Sum**	0.8 ± 1.5	1.5 ± 2.2	4.6 ± 5.6	3.2 ± 4.6
	BVAQ	**Analyzing**	8,8 ± 3.0	9.1 ± 3.1	8.4 ± 2.7	10.1 ± 3.3
		Emotionalizing	9.6 ± 2.8	13.5 ± 3.2	9.0 ± 1.5	12.2 ± 3.0
		Fantasizing	10.3 ± 4.1	11.5 ± 4.2	8.1 ± 4.2	9.6 ± 4.3
		Identifying	7.8 ± 2.0	7.4 ± 2.4	7.2 ± 2.2	9.6 ± 3.7
		Verbalizing	8.4 ± 2.6	11.2 ± 3.7	8.2 ± 3.3	12.7 ± 3.9
Time between scans [days]			158 ± 36	133 ± 15	138 ± 27	152 ± 46

2013; Peters et al., 2016). At rest, also the default mode network (DMN) was suggested to be highly relevant for self-referential mental activity (Davey et al., 2016). As reported by Uribe et al., the interaction of these networks differ between cisgender and TX cohorts pointing towards an implication in one's own gender identity perception (Uribe et al., 2020). As an essential component of both the CAN as well as the SN, the insular cortex is functionally implicated in interoception (Craig, 2002; Zaki et al., 2012). Although the SN was shown to work independently of the DMN, co-activation patterns were demonstrated between the posterior insula and the DMN (Harrison et al., 2011). In contrast to some prior reports were no significant influence of GHT on the DMN and the SN was detected (Nota et al., 2017), we found an increase in effective connectivity of the insula (which is part of the SN) to the left AG at rest in TW when compared to CM after GHT.

The AG, one of the major connecting hubs within the human brain, is implicated in many cognitive functions (Fjell et al., 2015). Interestingly, Burke and colleagues found significantly higher AG-activation in healthy adults while performing the so-called body localizer task. This was implemented to localize brain regions that are active while pictures of the own and other human bodies are presented and processed (Burke et al., 2019), suggesting a role of the AG in visual body perception. Own-body perception is a highly important aspect in the context of gender dysphoric patients. The significant increase of effective connectivity between the insula and the left AG among TW in comparison to CM might points towards a considerable influence of long-term estrogen treatment and accompanying physical and emotional changes to own-body-perception in this subgroup.

Additionally, significantly higher effective connectivity between the left MFG and the PC was shown. With connections to the CAN, the PC is one of the core interoceptive areas of the human brain (Benarroch, 1993). For instance, de la Fuente et al. found that grey matter volume of the left PC positively correlated with interoceptive accuracy (de la Fuente et al., 2019). Interestingly, we also found a decrease in effective connectivity, namely in the PC as well as the left AG towards the left MFG and from the right insula to the left MFG in TW when compared to CM. The connectivity changes between these regions after GHT in TW may be interpreted as a balancing communication pattern within the DMN. On the other hand, altered inter-network-communication pathways after GHT could be discussed between the DMN, CAN and SN in response to feminizing GHT and other aspects of transition in TW. Interestingly, not only feminizing, but also masculinizing GHT seems to influence the aforementioned networks. As reported by Burke et al., testosterone treatment in TM resulted in increased connectivity between the medial prefrontal cortex and the temporo-parietal junction in comparison to cisgender controls (Burke et al., 2017).

As mentioned, these effects were detected after GHT, suggesting an influence of feminizing GHT on selected regions and networks within the brain that are centrally involved in interoceptive processing. Interoception is an aspect of paramount importance in the treatment of gender dysphoria due to its relevance for own-body perception. For instance, Feusner et al. investigated the relation of DMN and SN activity to self-perception ratings using images of the subject's own body that were modified towards the opposite sex (Feusner et al., 2017a). The results pointed towards altered connectivity within networks involved in own-body perception in the context of self in GD. However, own-body perception in particular and interoception in general are not only relevant for GD, but seem to play a crucial role in a multitude of psychiatric diseases, e.g., anorexia nervosa (Pollatos et al., 2008) or major depression (Khalsa et al., 2018). These conditions show higher prevalence rates in females and phenotypic differences are well documented (Afifi, 2007) with women paying more attention to interoceptive sensations then men (Grabauskaitė et al., 2017). Women's higher attention to interoceptive stimuli could be, at least in part, discussed as a phenomenon resulting from higher estrogen levels in comparison to men. Thus, the increase in effective connectivity after feminizing GHT in TW across brain regions that are centrally involved in processing interoceptive stimuli might result from higher estrogen levels after GHT. Nevertheless, such causal interpretations need to be handled critically as GHT is not the only measure taken in the course of transition. Additionally, we did not perform any kind of in-vivo molecular analysis (such as positron emission tomography) to quantify cerebral hormone levels or, e.g., estrogen receptor density. In future studies, the interaction of hormonal changes in the brain and their relation to interoception could be of interest when exploring the physiologic mechanisms behind sex differences and the impact of exogenously administered sex hormones. Thus, although not assessed in this study, the effect of estrogen treatment on a molecular level deserves some consideration and could be addressed in future in-vivo functional molecular imaging studies. The distribution of estrogen receptor subtypes and the effect of estrogen on, e.g., dopaminergic and glutamatergic neurotransmission (Morissette et al., 2008) might have contributed to increased or decreased connectivity between certain brain regions after feminizing GHT. Interestingly, especially the presence of ERβ seems to be highly relevant for defeminization (Kudwa et al., 2005) and the effects of estrogen in the brain. Cerebral ERβ distribution might also play a role for the reported connectivity changes and thus the effect of exogenously administered estrogen in TW.

In our sample, the long-term administration of estrogens and androgen blockers in TW resulted in altered connectivity patterns of the insula as well as other brain regions that are implicated in DMN activity. However, it should be taken into consideration that due to clinical needs, different types of GHT administration were used within the same cohorts (i.e., both in the TW and in the TM group) and indirect effects of additional progestins and GnRH-analogues as well as of varying application forms of androgens and estrogens could not be ruled out completely.

Another aspect worth to be discussed is the change in alexithymia among TW after GHT. Alexithymia means a reduced awareness for emotional states (Taylor, 2000) and has been discussed as the consequence of impaired interoception and own-body perception (Gu et al., 2013), processes that are known to differ between men and women as well as between cis- and transgender cohorts (Feusner et al., 2017b).

The BVAQ was developed to analyze the five dimensions of alexithymia, namely "emotionalizing", "fantasizing" (about virtual matters), "identifying" (the nature of one's own emotions), "analyzing" (one's own emotional states) and "verbalizing" (one's own emotional states) (Vorst and Bermond, 2001).

In the present study, an increase in the identifying factor and a decrease in the fantasizing factor of the BVAQ among TW after GHT were found. In contrast, Mazzoli and colleagues found a general decrease in alexithymia among gender dysphoric individuals after GHT (Mazzoli et al., 2022). In the latter study, the Toronto Alexithymia Scale (TAS) (Bagby et al., 1994) was used to measure alexithymia. In our sample, two dimensions changed in opposite directions. This might be, at least in part, explained by certain factors that were not assessed in this study, such as the influence of complementary measures during transition (e.g., psychotherapy or social transition). In summary, these results show that transition involving long-term GHT has some effect on the ability to identify emotions. Since the increase in the identifying factor was limited to TW, especially exogenous estrogen treatment and androgen suppression might influence alexithymic traits to a higher extent than masculinizing GHT. Nevertheless, the results of this exploratory analysis should be taken with caution.

As stated by Chong and coworkers, the functional heterogeneity of the insular cortex and its activation patterns both in task-based approaches as well as resting-state analyses point towards a role as an interface coordinating the interpretation of and reaction to external stimuli involved in general cognition as well as affective and sensorymotor processing (Chong et al., 2017). The alterations in effective connectivity comprising the insula, the left AG, the left MFG, and the PC might be a result of the measures that were taken throughout transition in TW, e.g., estrogen and anti-androgen treatment in the scope of GHT. Although no assessment of language skills has been performed in this

study, the transition process involving long-term GHT might affected neuronal circuits involved in a variety of cognitive tasks, e.g., language processing. Here, we suggest that the long-term administration of estrogen resulted in an increase of effective connectivity at rest within certain networks known to be involved in verbal memory and linguistic processing (Ardila et al., 2014). Our findings are in line with results from an fMRI study investigating cerebral activation in language processing, where Sommer et al. found that total language activity correlated with estrogen levels after GHT (Sommer et al., 2008). Sex differences in various domains of language use and processing were discussed (e.g., Cherrier et al., 2003; Hyde and Linn, 1988), and the effect of exogenous estrogen on these skills might be an interesting research target for future transgender studies in view of the present data.

Since the abovementioned connectivity changes over time were only true for TW, but not for CW, it could be hypothesized that exogenously administered estrogen or androgen blockers enhanced synaptic plasticity in a multimodal manner. However, further studies need to be done to explore the underlying mechanisms, e.g., whether estrogen administration affects endothelial receptor density (Khalil, 2013), grey matter volume (Holmes, 2016), white matter microstructure (Kranz et al., 2017), alterations of intra-cerebral estrogen synthesis (Hojo et al., 2008) and the interaction with certain neurotransmitter systems such as the serotonergic system (Kranz et al., 2014).

4.1. Limitations

The results presented here should be seen in light of some limitations. Due to recruitment difficulties, TX subgroups were considerably imbalanced. Even though no significant longitudinal changes in HAM-D24 and WHOQOL-BREF were found, it cannot be ruled out completely that the transition period was accompanied by subclinical mood changes and/or altered own-body perception that contributed to the interaction effects presented here. Furthermore, it needs to be taken into account that language skills have not been assessed in this study. Based on the connectivity changes described here, GHT seems to affect brain regions and functional networks implicated in language processing. Therefore, TX individuals undergoing GHT might be an interesting cohort for future studies in which language skills are assessed before and after GHT.

5. Conclusion

The present study revealed that after long-term estrogen administration and anti-androgen treatment in the context of GHT, TW showed significant changes of resting-state connectivity of the right insula with the left AG, left MFG and PC when compared to CM. This suggests a considerable influence of GHT on brain networks implicated in interoception, own-body perception and higher-level cognition needed for e.g., language processing. Investigations considering the underlying mechanisms on a molecular level are needed to sufficiently understand the interplay of GHT, gender dysphoria and functional connectivity.

CRediT authorship contribution statement

Murray B Reed, MSc; conceptualization, methodology, software, formal analysis, data curation, visualization, writing – original draft, **Dr. Patricia A Handschuh, BA;** conceptualization, investigation, visualization, writing – original draft, **Manfred Klöbl, MSc**; investigation, data curation, writing – review & editing, **Dr. Melisande E Konadu;** investigation, writing – review & editing, **Dr. Ulrike Kaufmann;** investigation, writing – review & editing, **Prof. Andreas Hahn, MSc, PhD;** software, supervision, writing – review & editing, **Prof. Georg S Kranz, MSc, PhD;** conceptualization, writing – review & editing, **Dr. Marie Spies, PhD;** conceptualization, investigation, writing – review & editing, **Prof. Dr. Rupert Lanzenberger;** project administration, funding acquisition, supervision.

Declaration of Competing 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. The other authors report no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2023.106336.

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