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Intrinsic network connectivity and own body perception in gender dysphoria

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Abstract

Gender dysphoria (GD) is characterized by incongruence between one's identity and gender assigned at birth. The biological mechanisms of GD are unclear. We investigated brain network connectivity patterns involved in own body perception in the context of self in GD. Twenty-seven female-to-male (FtM) individuals with GD, 27 male controls, and 27 female controls underwent resting state fMRI. We compared functional connections within intrinsic connectivity networks involved in self-referential processes and own body perception – default mode network (DMN) and salience network – and visual networks, using independent components analyses. Behavioral correlates of network connectivity were also tested using self-perception ratings while viewing own body images morphed to their sex assigned at birth, and to the sex of their gender identity. FtM exhibited decreased connectivity of anterior and posterior cingulate and precuneus within the DMN compared with controls. In FtM, higher “self” ratings for bodies morphed towards the sex of their gender identity were associated with greater connectivity of the anterior cingulate within the DMN, during long viewing times. In controls, higher ratings for bodies morphed towards their gender assigned at birth were associated with right insula connectivity within the salience network, during short viewing times. Within visual networks FtM showed weaker connectivity in occipital and temporal regions. Results suggest disconnectivity within networks involved in own body perception in the context of self in GD. Moreover, perception of bodies in relation to self may be *reflective* rather than *reflexive*, as a function of mesial prefrontal processes. These may represent

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neurobiological correlates to the subjective disconnection between perception of body and self-identification.

Keywords

gender identity disorder; transsexual; transgender; body image; body identification; resting state fMRI

Introduction

Gender dysphoria is often theorized to be a consequence of atypical cerebral sexual differentiation (Swaab 2004). However, brain imaging studies, while being consistent with respect to structural and functional differences among male and female controls, are far less consistent in demonstrating evidence of atypical or divergent sexual differentiation in the brains of those with gender dysphoria. In the quest for better understanding of the neurobiology of GD it is important to explore the basic symptoms and fundamental subjective experiences associated with this condition. Gender identity disorder (GID), Transsexualism ICD 10 (WHO 1992) referred to as gender dysphoria (GD) in DSM-5 (American Psychiatric Association, 2013), is characterized by a stark feeling of incongruence between one's gender assigned at birth and the gender that they identify with. Body dysphoria and body-related avoidance (e.g., not looking in the mirror or hiding one's body under baggy clothes) (American Psychiatric Association 2013; Cohen-Kettenis and Pfafflin 2010; Coleman et al. 2012) have been found to be key features of GD, emerging from a strong perception of incongruence between one's sense of self and one's body. The perception of own body has been conceptualized as molded by a reciprocal interaction between sensory perceptions of one's physical appearance, experiences based on self observation and reactions of others (Cash and Pruzinsky 2002), and own body image in the brain (Vocks et al. 2010).

An important issue in the neurobiology of GD which has hitherto received relatively scant attention is whether GD could be associated with specific patterns of body perception, and if so, whether this is linked to functional neural signatures in certain cerebral networks. One theoretical possibility, which is also hypothesized in the present work, is that in GD individuals the typical physical traits of their gender assigned at birth are not incorporated into their self-representation, and that this is associated with specific functional connectivity signatures in the brain.

The concept of own body perception in the context of self

Neural representation of the self is often viewed as being comprised of physical and psychological aspects (Gillihan and Farah 2005). Physical aspects of the self have been examined via self-face recognition, body recognition, agency, and perspective taking, while psychological aspects of the self have been examined using autobiographical memory and self-knowledge or self-referential processing of personality traits (Istvan Molnar-Szakacs and Uddin 2013). Neuroimaging studies suggest that own body perception, vs. psychological or self-referential processes, rely on distinct, yet interacting large-scale brain networks (I.

Molnar-Szakacs and Arzy 2009; Uddin et al. 2007). Previous studies suggest that physical aspects of the self such as recognition and identification of body as own may be mediated by the right inferior parietal lobe and the temporo parietal junction (TPJ), precuneus and posterior cingulate, posterior orbital gyrus, and the right insular cortex (Northoff and Panksepp 2008; Devinsky et al. 1989). All may interact with systems that have been found to be involved in visual processing of bodies such as extrastriate and fusiform body areas (Paul E. Downing and Peelen 2011; P. E. Downing et al. 2007; Peelen and Downing 2007). This is based on data from functional magnetic resonance imaging (fMRI) experiments comparing viewing own body and familiar bodies (Hodzic et al. 2009; Vocks et al. 2009). Furthermore, damage to, or seizures originating from, the right angular gyrus (part of the TPJ) has been observed to lead to out-of-body experiences (Heydrich et al. 2011; Blanke et al. 2004; Devinsky et al. 1989). Self-referential processing, (reflecting the psychological aspects of self), incorporates perception of own body in the context of self. This consistently involves the pregenual anterior cingulate cortex (pACC), as demonstrated in a quantitative meta-analysis (Northoff et al. 2006).

Resting state fMRI and intrinsic connectivity networks in relation to own body perception in the context of self

A plethora of resting state fMRI studies have measured temporal correlations of fluctuations of the BOLD signal, which is thought to reflect synchronous neuronal activity (see, for example (Biswal et al. 1995; Greicius et al. 2003a; Beckmann et al. 2005; Fox et al. 2005)). Many of the networks showing high intrinsic connectivity at rest are those that activate during specific tasks; Performance of these tasks outside the scanner has been found to correlate with the intrinsic resting state connectivity of the network involved in processing this task (Cole et al. 2014).

The default mode network (DMN) is an intrinsic connectivity network that previous studies have shown to be involved in mind-wandering and self-referential thinking (Christoff et al. 2009; Mason et al. 2007; Northoff and Panksepp 2008). The DMN is found to overlap with areas involved own body perception in the context of self but has received little attention in studies of GD. It is comprised primarily of the PCC, medial prefrontal cortex (mPFC), and lateral parietal cortices (Raichle et al. 2001; Greicius et al. 2003b). Another network theorized to be involved in own body perception in the context of self is the salience network (SN), consisting of the fronto-insular and anterior cingulate cortices (Seeley et al. 2007). The SN has been found to be involved in processing and evaluating various signals from own body, including interoceptive states (Craig 2002) and stimuli that have social and emotional relevance (Uddin 2015).

Examinations of functional brain networks underpinning own body perception in the context of self have thus far not been performed in GD. However, we recently found that cortical thickness is increased in GD compared with both male and female controls, specifically along the midline axis (pACC, the superior, rostral, and middle frontal gyri – including portions of the orbitofrontal gyrus), in occipital regions (right lingual gyrus, pericalcarine cortex, and cuneus), and in the right inferior temporal gyrus (Manzouri et al. 2015). This raises the question whether the functional connectivity within the DMN (which includes

pACC), and/or other networks involved in self- and own-body awareness (SN) and perception (visual networks) could differ in GD from controls, and if distinct patterns of connectivity could be related to perception of own body perception in the context of self. That neuronal circuitry mediating own body perception in the context of self may be involved in GD is also suggested by an earlier study by our group, carried out in male-to-female transsexuals and showing increased grey matter volume in the TPJ (Savic and Arver 2011).

To investigate this issue we acquired resting state fMRI data in female-to-male (FtM) GD individuals and female and male controls. We hypothesized that connectivity within the DMN would be abnormal in FtM, given the finding of greater Cth in the pACC (Manzouri et al. 2015). Further, we predicted connectivity in this network would be weaker in FtM than in both control groups, following a model of lesser integration of own-body representation of physical traits of their gender assigned at birth (posterior division of DMN) and their consciously accessible, “psychological” self-representation (anterior division of DMN, as shown in (Salomon et al. 2014)). In sensory integration systems (insula, as part of the SN) and primary sensory perception systems (visual networks) we hypothesized that there would be no group differences in connectivity patterns. This would also be consistent with a model of differences primarily in higher-order conscious self-representation, rather than sensory perception and integration, which is consistent with findings in our previous study examining behavioral responses to visual images of their own body (Feusner et al. 2016).

In addition, we investigated the relationships between network connectivity in DMN, SN, and visual networks, and perception of self in relation to own body and bodies congruent and incongruent with their gender identity. To this end, we used a “body morph test” (see (Feusner et al. 2016), Fig. 1, and Methods). Furthermore, we investigated whether body identification is linked to a more “reflective” complex decision-making process (as permitted by longer viewing times), or if this phenomenon is more perceptually driven and “reflexive,” and therefore occurring with short viewing times. A reflexive response would be indicative of more immediate recognition, which is faster for self vs. others and for intact vs. altered images (both of bodies and faces) (Devue et al. 2007; Verosky and Todorov 2010; Devue and Bredart 2011). A reflective process, on the other hand, would be one that involves cognitive factors that are conscious and goal-directed, and/or may involve making a decision, and therefore would likely be on a longer time-scale (Verosky and Todorov 2010; Henderson 2007). We hypothesized that FtM (but not controls) would demonstrate significant associations between connectivity and self-body perception within the DMN for long viewing times. This is based on the observation in our previous study that FtM persons, when given longer duration to view morphed bodies image showed higher self-identification ratings towards the sex they desired to be, consistent with a more reflective process involving conscious, self-referential thinking (Feusner et al. 2016). In this study shorter duration of viewing times was associated with ratings closer to what was observed in controls; thus, we predicted that short viewing times in FtM and controls would show similar associations with visual and SN connectivity involved in earlier perceptual and sensory-integrative systems, as the more reflexive self-processing was predicted to be similar in the two groups.

Materials and Methods

Participants

Twenty-seven female-to-male (FtM) persons with GD (24.2 ± 5.4 years, range 18-34 years, education 13.1 ± 2.7 years, range 9-18 years), 27 heterosexual male controls (HeM; 31.0 ± 6.1 years, range 20-39 years, education 16.2 ± 2.45 , range 12.5-21 years), and 27 heterosexual female controls (HeW; 32.1 ± 7.3 years, range 20-32 years, education 13.1 ± 2.7 years, range 12-21 years) participated. None of the FtM persons had received hormone treatment or sex confirmation surgery (also called sexual reassignment surgery). The FtM persons were recruited by the Gender team at the Karolinska University Hospital (Stockholm, Sweden) specialized for the evaluation and treatment of persons with GD. All consecutively arriving adults aged 18 - 45 years who sought gender confirming medical interventions, and were diagnosed with GD, specifically transsexualism (F64.0) based on the ICD-10 diagnostic criteria (<http://www.who.int/classifications/icd/en/>) were approached to enter the study, between January 2011 and November 2014. Exclusion criteria consisted of previous or current hormonal treatment, any known chromosomal or hormonal disorder, any current psychiatric disorder (as confirmed by the Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al. 1998), any neurological or other major medical disorder, or any medications with psychotropic effects (antipsychotic or antiepileptic agents, lithium, benzodiazepines or opioid analgesics).

All the participants scored their sexual orientation using the Kinsey (Heterosexual-Homosexual Rating) scale (Hahn et al. 2015) seven-point scale ranging from 0 to 6. The approach is described in detail in previous studies (Shott et al. 2016; K. R. Kim et al. 2012).

Because there is a considerable comorbidity between GD and autism spectrum disorders (ASD), (de Vries et al. 2010), we specifically excluded participants with known ASD (diagnosed before being referred to the team) or participants who showed clinical signs of ASD when being assessed by the team, which lead a careful and specific evaluation for ASD). All included GD participants were also investigated for presence of autistic traits with The Social Responsiveness Scale (SRS) (Gardner and Brown 2014). The SRS was administered to all GD participants and required that someone close to them, such as parent, partner or close friend, complete it. The 65-item SRS questionnaire inquires about autistic behavior over the previous 6 months is scored from 0 (never true) to 3 (almost always true). Higher points on the questionnaire signify more severe autistic traits.

Controls consisted of healthy individuals without any neurological or psychiatric illnesses or family history, no substance abuse, or ongoing medication. The study was approved by the ethical committee at the Karolinska Institute and each participant provided signed consent before entering to the study.

Magnetic Resonance Imaging and resting state image processing

Magnetic resonance imaging data was acquired on a 3-Tesla MRI medical scanner (Discovery 3T GE-MR750, General Electric, Milwaukee, Wisconsin) equipped with a 32-channel phased array receiving coil. Resting state functional MRI was performed with a gradient echo pulse sequence using a voxel size of $2.25 \times 2.25 \times 3$ mm, (TE=30ms,

TR=2500ms, FoV=28.8cm, 45 bottom up interleaved axial slices, 3mm thickness, flip angle of 90 deg). 3D T1-weighted SPGR (Spoiled Gradient Echo pulse sequence) images were acquired with 1 mm³ isotropic voxel size (TE=3.1ms, TR=7.9ms, TI=450ms, FoV=24cm, 176 axial slices, flip angle of 12 degree).

Initial preprocessing of the functional images was performed using SPM8 (Wellcome Department of Cognitive Neurology). Functional images were slice-timed and realigned using the 'Realign and unwrap' function, incorporating fieldmap correction, and then registered to structural T1-weighted SPGR images for each participant. Next, the individual T1-weighted SPGR images were segmented into gray matter, white matter, and cerebrospinal fluid, and the gray matter image was used to determine the parameters of normalization for the standard Montreal Neurological Institute gray matter template. The spatial parameters were then applied to the slice-timed and realigned functional volumes that were finally resampled to 2 × 2 × 2 mm voxels and smoothed with a 6-mm full-width at half-maximum kernel.

Independent components analysis

Further pre-processing and statistical analyses were carried out in FSL v5.0 (FMRIB Software Library, Oxford, <http://fsl.fmrib.ox.ac.uk/>). We used an independent components analysis (ICA) approach because, as a multivariate, data-driven procedure it can separate artifact from true brain activity-related fMRI signal (Xu et al. 2013; McKeown et al. 1998; Calhoun et al. 2001; Malinen et al. 2007; Tie et al. 2008; D. I. Kim et al. 2009). In addition, it allowed us to isolate the DMN, SN, and visual networks of interest, using a data-driven approach based on functional activation rather than structural anatomy. This is particularly useful for resting state fMRI data where the time course of the response is uncertain. The preprocessed images at this stage were high-pass filtered at 100s using `fslmaths` to remove slow scanner artifacts before being subjected to individual ICA, using Probabilistic ICA (Beckmann and Smith 2004) as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components, Version 3.14), with automatic determination of dimensionality. The resulting component maps were then manually classified into components of interest and nuisance-components in accordance with the criteria proposed in (Kelly et al. 2010). The components marked as nuisance were subsequently regressed out of the original data set using `fsl_regfilt`. This allowed us to remove components that were generated by noise such as scanner artifacts or motion.

The following additional pre-processing was applied to the input data: masking of non-brain voxels; voxel-wise de-meaning of the data; and normalization of the voxel-wise variance. Pre-processed data were whitened and projected into a n-dimensional subspace using probabilistic Principal Component Analysis where the number of dimensions was estimated using the Laplace approximation to the Bayesian evidence of the model order (Beckmann and Smith 2004). The whitened observations were decomposed into sets of vectors, which describe signal variation across the temporal domain (time-courses), the session/subject domain and across the spatial domain (maps) by optimizing for non-Gaussian spatial source distributions using a fixed-point iteration technique (Hyvarinen 1999). Estimated component

maps were divided by the standard deviation of the residual noise and thresholded by fitting a mixture model to the histogram of intensity values (Beckmann and Smith 2004).

Group concat-ICA was performed on the whole cleaned dataset resulting in 22 components. These components were used to run dual-regression and the resulting GLM parameter estimate images were fed into FSL's Randomise nonparametric permutation inference, in order to test the separate hypotheses about differences among groups in connectivity within the DMN, SN, and visual networks. The group ICA procedure, followed by dual-regression, allows for slight individual variations in spatial location of activation in individual subject space, again likely increasing sensitivity as it avoids errors and loss of signal that can occur when registering to a common space. The design included age and mean DVARS (Root Mean Square intensity difference of volume N to volume N+1 (Power et al. 2012), which indexes effects of motion, as nuisance covariates. Although there are several indexes of motion to potentially use as covariates (e.g. framewise displacement or FD), we chose DVARS as it accounts for actual changes in image signal quality that occur with motion and not just the motion-related displacement (Power et al. 2012). The significance threshold was set at $p < 0.017$, FWE corrected, to account for three pairwise group comparisons: 1) FtM-TR vs. HeW, 2) FtM-TR vs. HeM, and 3) exploratory testing of HeW vs. HeM.

Body-morph test

Participants, outside of the scanner, viewed photographs of their bodies morphed to varying degrees towards other female bodies (Fig.1). This allowed us to obtain ratings that index own body identification with images of their body that are morphed to appear more masculine or more feminine. Furthermore, we investigated whether body identification is linked to more reflective, complex and therefore time consuming cognitive factors such as decision-making process (as permitted by longer morphed body viewing times of 2 seconds), or if this phenomenon is more perceptually-driven and reflexive, and therefore occurring with short morphed body viewing times of 0.5 seconds. We predicted that short viewing times would primarily be associated with visual and salience network connectivity involved in earlier perceptual (reflexive) and sensory-integrative systems.

The body morph test was conducted outside of the scanner in 15 of the controls (eight males, age 24.5 ± 4.6 years, seven females, age 24.6 ± 8.2 years) and 18 of the FtM (age 24.1 ± 6.0 years) – only a portion of the total sample performed the body morph test, as this was added after MRI data collection had begun. For details of the body morph test, please refer to (Feusner et al. 2016). Each participant's photograph was morphed separately with pictures from three different HeW and three different HeM, from the front and side, to degrees of .20, .40, .60, .80, and 1.00 (Fig.1), using FantaMorph Software version 5.0 (Abrosoft <http://www.fantamorph.com/>). Note that the 1.00-degree photos were entirely images of another person. We also included a front-view and a side-view unmorphed picture of each participant. The 62 unique morphed or unmorphed images depicting the participant's body were randomly presented on the computer screen, for either 2 or 0.5 seconds (short vs. long duration). Participants were instructed to respond as quickly as possible after each image to the question, “to what degree is this picture you?” by pressing computer keys 1 to 4 (1: “0-25% me,” 2: “25-50% me,” 3: “50-75% me,” or 4: “75-100% me”).

We next calculated an ‘own body perception index’ by multiplying each degree (1-4) of “self” rated for each morph with the degree of each morph. Positive values indicated morphs to their sex assigned at birth for both groups; Negative values indicated morphs to the opposite sex for controls, and the sex congruent with their gender identity for FtM. These values were averaged across ratings for all images and divided by the number of rated images, providing an average index of self-perception for each participant weighted by how close or far from the actual self-photograph the image was morphed, and in which direction. This ‘own body perception index’ was entered as covariate in the same intrinsic resting state connectivity components of interest (DMN, the SN and the visual network), within each group. To ensure specificity to regions within these networks, we used masks composed of the respective group components to correspond to the previously defined networks (Shirer et al. 2012). We used a significance value of $p < 0.025$, to account for within-group covariation analyses for FtM and (combined) male and female controls, FWE corrected.

Results

Demographics

There was no age or educational difference among HeW and HeM. FtM were younger and had less years of education than controls (Table 1). (Because of significant group differences in age, we used age as a covariate in the between-groups comparisons – see Methods, Independent Components Analysis.) Adding education as covariate did not significantly change the results; therefore we decided not to include in our final model to conserve power and maximize stability of the estimates. The sexual orientation was heterosexual in both control groups. The FtM persons predominantly reported gynephilic (attraction to females) preference (Table 1). The testosterone and oestrogen levels in participants with GD were within the normal range (Table 1), congruent with the notion that they were investigated before receiving any hormone treatment.

Behavioral Results: Body morph index ratings

FtM showed mean body morph index ratings more towards the opposite sex than controls for long exposures (-12.54 ± 16.28 vs. 12.16 ± 12.39) and short exposures ($-.30 \pm 9.30$ vs. 13.44 ± 17.20), which was reflected in a significant group effect ($F(1, 30) = 28.52, p < .001$, partial $\eta^2 = .49$). There was a trend level effect of viewing duration ($F(1, 30) = 3.82, p = .060$, partial $\eta^2 = .11$), indicating ratings more towards the same (assigned at birth) sex with shorter viewing durations. There was not a significant group by viewing duration effect ($F(1, 30) = 2.50, p = .12$, partial $\eta^2 = .077$).

No group difference was detected in movement as indexed by DVARS values (HeM 5.97 ± 1.21 ; HeW 5.81 ± 1.34 ; FtM 5.37 ± 1.01 ; one-way ANOVA: $F(2,81), p = 0.160$).

Group difference in connectivity patterns within the DMN

FtM showed weaker connectivity than both HeW and HeM within DMN in the ACC, PCC and precuneus (Fig. 2, Table 2). There were no differences between HeW and HeM, and no regions with stronger connectivity in FtM than controls.

Group difference in connectivity patterns within the SN

No significant group differences in connectivity were detected in the SN.

Group difference in connectivity patterns within the visual network

FtM showed weaker connectivity than HeW in the right cuneus, lateral occipital cortex superior, right and left occipital poles, left occipital fusiform, left lingual gyrus, left temporal occipital fusiform gyrus, and left intracalcarine and supracalcarine cortices (Fig. 3, Table 2). FtM showed weaker connectivity than HeM in right lateral occipital cortex, although not surviving correction for multiple comparisons ($p=.034$, FWE corrected). Additionally, there was a cluster with stronger connections in FtM compared with HeW in left lingual gyrus. There were no significant differences between HeW and HeM.

Body morph index results in relation to covariation patterns in DMN

In FtM body morph index scores were negatively associated with connectivity in the pACC/paracingulate gyrus (cluster size 116 voxels; local max $x=-12$, $y=52$, $z=4$; $p=.006$, FWE corrected). Thus, the more the GD individuals perceived their body as the sex they desired to be, the stronger was the connectivity within the left pACC (Fig. 4a). This covariation was present only for the long viewing duration (2 sec). No significant covariation between the body morph index and DMN connectivity was detected in controls.

Body morph index results in relation to covariation patterns in SN

Controls displayed a significant positive association between the body morph index and SN connectivity in the right insula, notably, during the short viewing duration (0.5 sec), Fig. 4b. Thus, the more the HeW and HeM self-identified with body images of the same sex, the stronger was the connectivity with right insula (cluster size 101 voxels; local maximum $x=34$, $y=-6$, $z=4$; $p=.015$, FWE corrected).

Body morph index results in relation to covariation patterns in visual network

No significant covariations were detected for FtM or controls between the body morph index and the visual network for either exposure time.

Discussion

In this study we combined resting-state fMRI and behavioral data to investigate the neurobiological underpinnings of GD. Previous brain imaging studies of GD have largely probed whether their brains are sexually differentiated in a manner that is opposite to their sex assigned at birth. In contrast to these, the present study investigated whether in GD individuals there are functional connectivity differences in networks involved in own-body perception in the context of self. We found that individuals with GD (specifically, FtM) had weaker connections within the DMN in the precuneus, PCC and in the ACC. In addition, they showed a pattern of rating 'self', when their body image was morphed to the sex congruent with their gender identity. During longer viewing times, this rating co-varied with connectivity in the anterior portion of the DMN, which is known to be involved in self-referential processing. This evidence of divergent network patterns in FtM compared with

both male and female controls (which did not differ from each other), suggests that their brain connectivity signatures are not merely on a continuum between heterosexual male and heterosexual female.

Behavioral results

Unsurprisingly, the FtM showed body morph index scores consistent with greater identification with bodies morphed towards the sex congruent with their gender identity, than controls. This was more pronounced when given longer time to view and respond to the images, which may be more consistent with elaborated, conscious thinking and decision-making (reflective) than an innate or perceptually-driven response (reflexive), as we previously found in a behavioral study (Feusner et al. 2016). Although there was not a significant interaction effect of group by viewing duration in the present study, this may have been due to insufficient power in this subsample, as there was a medium effect size (partial $\eta^2 = .077$).

The own body perception network and the observed differences between FtM and controls

The concept of own-body as part of self is an integrative process (Moseley et al. 2012) requiring a complicated interplay of several structures, which are primarily located along the antero-posterior axis of the brain, and incorporate a parieto-occipital *body-detection* network and a fronto-parietal *self body-identification* network (Northoff and Panksepp 2008; Hodzic et al. 2009; Vocks et al. 2009; Devinsky et al. 1989) and a fronto-parietal *self body-identification* network (Northoff et al. 2006). In light of these data it is of interest that our GD cohort showed weaker functional connections within the self-body identification network indexed by significant differences from both male and female controls in the ACC and precuneus/PCC region of the DMN. Furthermore, their perception of own body as 'self' covaried with the pACC connectivity within the DMN, yet this covariation was evident only for the long viewing duration. One plausible interpretation of these findings is that in GD individuals there is a functional disconnection between subcomponents of DMN mediating perception of own body and reference to it as 'self' (Salomon et al. 2014). Moreover, the fact that their perception of own body as 'self' was higher for images morphed to the sex congruent with their gender identity and opposite to the sex they were assigned at birth suggests a process tying together body perception with self-identification that might be more reflective than reflexive, given that covariation with left pACC was present only for longer viewing durations. Longer durations would have allowed for more conscious, cognitive evaluation (Verosky and Todorov 2010; Henderson 2007). At variance from GD, in controls the index for perception of their morphed body as 'self' covaried with the right insular cortex within the SN, and only during short viewing durations. The SN is tightly coupled to the DMN, and is believed to be involved in processing and evaluating various signals from own body, including interoceptive states, in a more reflexive way than the DMN (Uddin 2015). The fact that this connectivity covariation pattern in the SN was not observed in GD suggests lesser participation of networks involved in more reflexive perception of own body. One possible explanation is that this could be an effect of top-down modulation in GD from midline prefrontal regions such as the pACC within the DMN on the salience network. Top-down modulation could also be operative in visual systems involved in body processing, accounting for the weaker occipital and temporal connectivity in GD compared with both

HeM and HeW (although only significant at our corrected threshold for the comparison with HeW). This would be in line with the theory that the DMN is a higher order cortical system that reciprocally exchanges information with subordinate brain systems (Carhart-Harris and Friston 2010).

An alternative is that the observed differences in functional connections may instead be simply a function of inherent anatomical differences between individuals with GD and controls. Indeed, our recent study of cortical thickness in a (partially overlapping) sample of GD individuals demonstrated significantly thicker cortex in the ACC, mPFC, precuneus, cuneus, left inferior parietal, and temporal cortex in FtM than both male and female controls (Manzouri et al. 2015). In the absence of longitudinal data it is, however, not feasible to disentangle between the two alternatives, which, in addition, are not necessarily mutually exclusive.

The present data do not support the hypothesis that sexual differentiation of the brain in individuals with GD is in the opposite direction as their sex assigned at birth; the weaker precuneus/PCC, and ACC connectivity within the DMN was detected in comparison with both male and female controls, and there were no differences between the two control groups. This accords with our structural MRI studies of GD (Manzouri et al. 2015; Savic and Arver 2011), as well as studies of white matter microstructure (Kranz et al. 2014), graph theoretical network analyses of white matter connectivity (Hahn et al. 2015), and resting state functional connectivity (Lin et al. 2014), all of which also suggest that GD may be associated with its own, singular features. In the latter resting state fMRI study, MtF and FtM as a group, compared with controls, demonstrated greater degree connectivity of the postcentral gyrus and superior parietal lobule (“body representation areas”) within a whole brain network, and greater connectivity of these nodes with sensorimotor network nodes. This study also found that the degree to which MtF and FtM identified with the desired gender, viewed in a video outside the scanner, was negatively correlated with connectivity between the right insula and right and left postcentral gyrus. In the current study, insula connectivity was associated with same sex identification in the controls but not the GD group. However, despite the design being somewhat similar to that in Lin et al. (2014) in the sense that both involved associating resting state connectivity with the subjective experience of viewing body images, the type of connectivity analyses (ICA vs. graph theory connectivity) and the networks interrogated (SN, DMN, and visual networks vs. whole brain connectivity and insula to postcentral gyrus connectivity) diverged, which could explain differences in results.

Methodological issues, and possible confounding factors

With respect to participant selection it is important to emphasize that all the GD participants were naïve to sex hormone treatment, and had estrogen and testosterone values which were within normal range for their sex assigned at birth, as shown in Table 1. In addition, the results cannot be attributed to psychiatric conditions, as this was a criterion for exclusion. Neither can they be explained by autistic traits, as assessed by the clinical evaluation and with SRS scores, which showed that GD participants did not differ from the general population (Table 1).

One might conceive that living with the perception of being “trapped in the wrong body” exposes GD individuals to a constant psychosocial stress, which, itself, could be associated with cerebral changes. Our recent studies show, however, that chronic stress is associated with a reduction of cortical thickness in the mPFC (Savic 2013), whereas FtM (in a sample partly overlapping with that in the current study) displayed increased cortical thickness in this region. Also, in chronically stressed individuals the cortico-cortical covariation (possibly indexing structural, and perhaps also functional, connectivity) between ACC and the insular cortex are increased (Savic 2013), which was not observed in the SN our GD sample. Moreover, concomitant significant depression or anxiety does not explain the present observations, as none of the participants fulfilled criteria for psychiatric disorders.

Sexual orientation of the participants is another factor to consider. Our GD participants rated on the homosexual end of the Kinsey scale in relation to their sex assigned at birth, whereas the controls were heterosexual. However, the pattern of functional connectivity in homosexual men and women is reported to be sex atypical (that is, to the opposite sex) (Savic and Lindstrom 2008), whereas the presently observed difference in functional connectivity in FtM persons was present in relation to both control groups, and thus unlikely to be a function of sexual orientation. Nevertheless, this aspect needs further evaluation in comparative studies between homosexual persons with and without GD.

The cross-sectional design of the study is a limitation that precludes establishing cause and effect in regard to possible etiology. Thus, we cannot determine if there is a direct causal relationship in which disconnections in DMN and visual networks lead to a propensity for diffuse or ambiguous self-identity, or diffuse or ambiguous self-identity over time leads to disconnections in DMN and visual networks. Post cross-hormone or surgical treatment analyses of functional connectivity, own-body perception, and cortical thickness in the same individuals might shed some light on this important issue. Another limitation of the study is that participants were not matched on age or years of education. However, we used age as a covariate in our analyses. In addition, we tested education as a covariate and it did not significantly change the results. An additional limitation of the present study was that, due to logistical issues, only a subset of GD was investigated with the body morph test. In addition, our GD group consisted of only FtM; without having also investigated MtF, the current results cannot necessarily be generalized to all GD populations. However, given that avoidance of viewing own body is a common behavioral feature of GD in general (Bandini et al. 2013; Cohen-Kettenis and Pfafflin 2010), it is possible that body identity is less crystalized. In this way, it is conceivable that across GD populations, their experience of not inhabiting their ‘true’ body leads them to develop a parallel body image towards the sex opposite to that assigned at birth. Additional studies across FtM and MtF are needed to further elucidate this interesting issue. Further, task fMRI studies using a body perception task would be able to more directly assess activation and connectivity patterns as they are engaged.

Conclusion

In conclusion, GD individuals differ from controls with respect to connectivity within networks involved in self-directed thinking and that relate to own-body identification, which

could represent a neurobiological correlate of their condition. Collectively, these convergent findings posit neurobiological associations with the self-thoughts and self-perceptions of GD individuals, at least in FtM. The data strengthen the notions that observable and measurable biological patterns are associated with gender identity, and that gender dysphoria is in the realm of human physiological variation. Whether this neurobiological marker varies among different populations of GD, if it is innate or acquired, and how it may be affected by sex hormone or surgical treatments are important issues to investigate in the near future.

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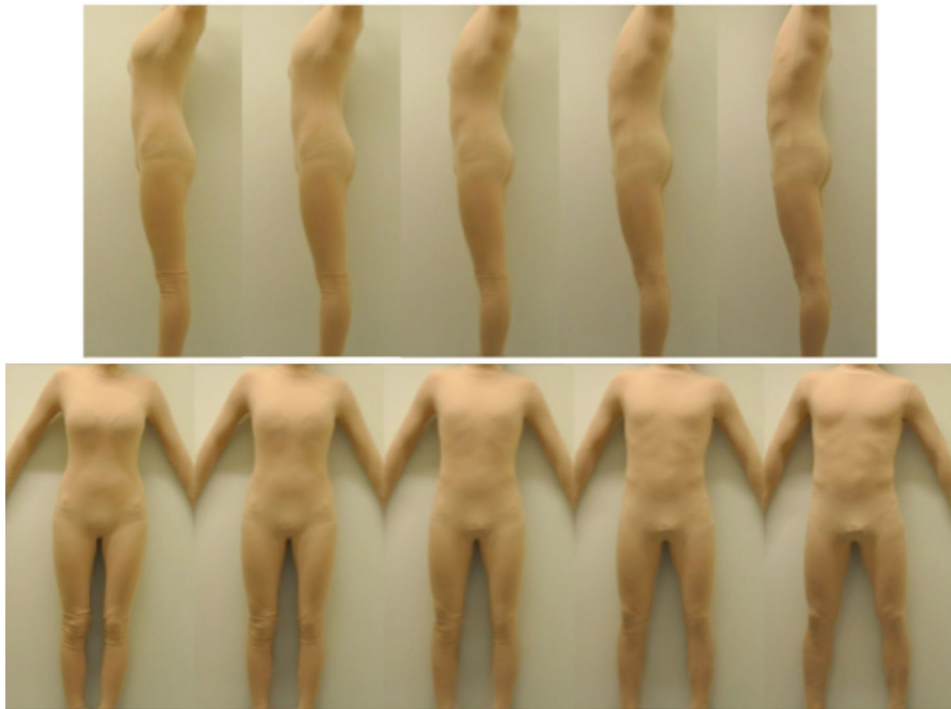


Figure 1. Example body morph images

Top row shows .20, .40, .60, .80, and 1.00 from left to right, morph to sex congruent with the gender identity, side view. Bottom row shows .20, .40, .60, .80, and 1.00 from left to right, morph to sex congruent with the gender identity, front view. Note that the 1.00 photos were entirely images of another person.

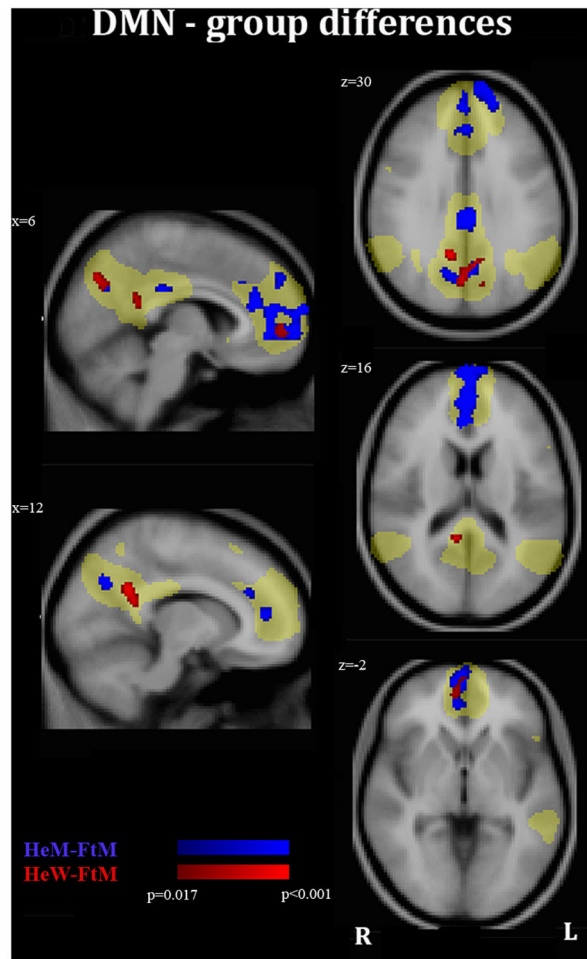


Figure 2. Group differences within the default mode (DMN) network. The mask for the DMN is indicated in yellow and superimposed on the mean T1 image of the entire group (in MNI space). Clusters showing stronger connections in HeM than in FtM are shown in blue; clusters showing stronger connections in HeW than in FtM are shown in red.

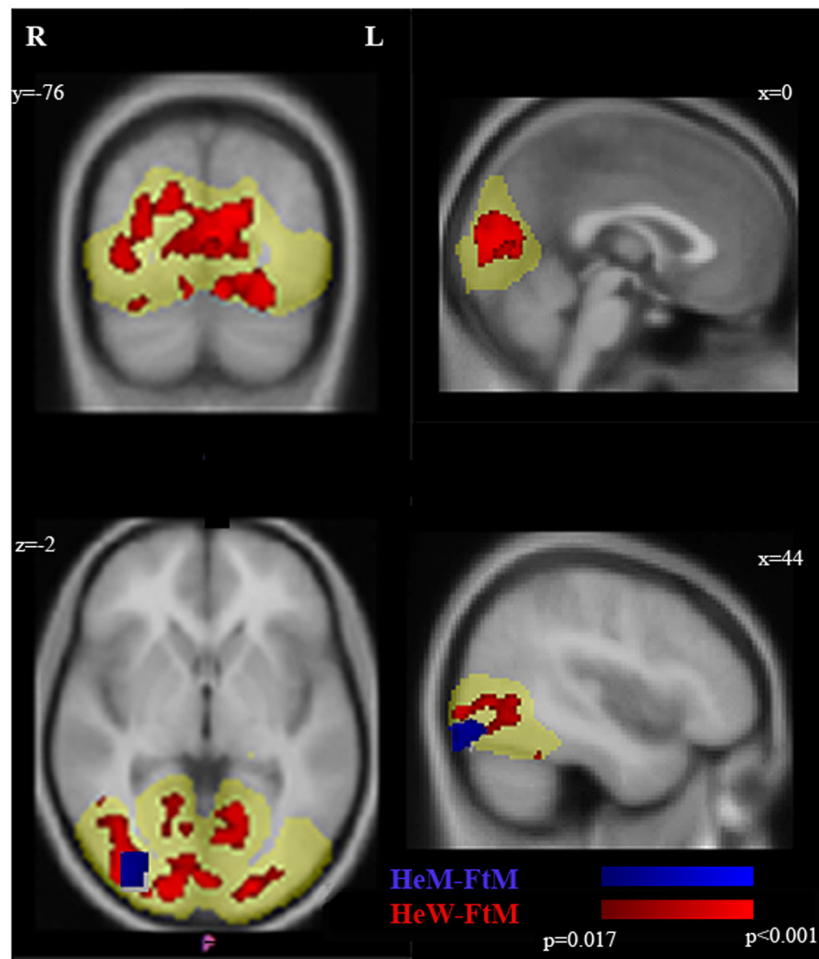


Figure 3. Group differences within the visual network. The mask for the visual network is indicated in yellow and superimposed on the mean T1 image of the entire group (in MNI space). Clusters showing stronger connections in HeM than in FtM are shown in blue; clusters showing stronger connections in HeW than in FtM are shown in red.

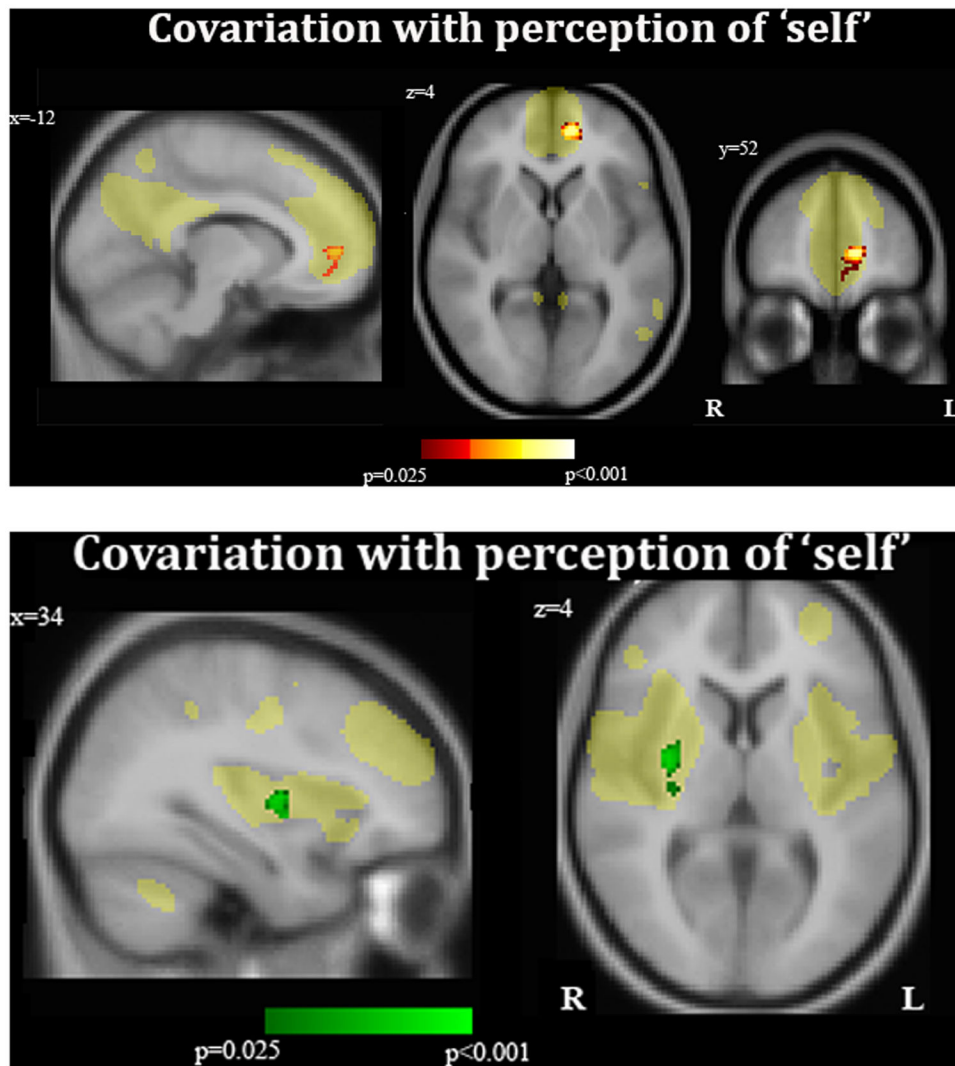


Figure 4. Associations between body morph self-identity ratings and connectivity within intrinsic networks

(a) Regions significantly associated in FtM with self-identity ratings of body images morphed towards the sex congruent with their gender identity for long viewing durations (hot red-yellow color), overlaid on the group default mode network (DMN) mask (yellow).

(b) Regions significantly associated in male and female controls with self-identity ratings of body images morphed towards the sex congruent with their gender identity for short viewing durations (green), overlaid on the group salience network (SN) mask (yellow).

Table 1

Demographic and hormone data of participants

	HeM N= 27		HeW N= 27		FtM N= 27		F-value ^f	P-value	
Unit	mean	SD	mean	SD	mean	SD			
Age	year	31.0	6.1	32.1	7.3	24.2	5.4	12.4	<0.001
Education	year	16.2	2.5	17.0	3.0	13.1	2.7	15.1	<0.001
Oestradiol (plasma)	pmol/L				520.7 [‡]	466.3			
Testosterone (total)	nmol/L				1.73	0.58			
Kinsey scale		0.48	0.9	0.41	0.6	4.3	1.9	82.1	<0.001
SRS score		43.5	5.9	46.3	9.1	48.7	9.1	2.4	0.096

^f F-values from group comparisons (one way ANOVA). FtM were significantly younger and less educated than both control groups, and had significantly higher Kinsey scores (higher at the homosexual end) than HeM as well as HeW. No plasma sex hormone levels were provided by controls.

[‡] In the FtM group there were no estrogen levels for two subjects, and six subjects had a level <150 Pmol/L. HeM = heterosexual male control individuals; HeW = heterosexual women control individuals; FtM = Female-to-male individuals with gender dysphoria

Table 2
Regions and local maxima for group differences in the default mode network (DMN) and visual networks

Group differences in the default mode network (DMN)							
HeM > FtM			HeW > FtM				
<i>Region</i>	<i>Cluster size (voxels)</i>	<i>x, y, z local maxima^a</i>	<i>p value</i>	<i>Region</i>	<i>cluster size (voxels)</i>	<i>x, y, z local maxima^a</i>	<i>p value</i>
ACC	2998	0, 44, 4	<.001	<i>Right ACC</i>	131	6, 54, 0	.026
Left precuneus	972	-2, -66, 30	.001	<i>Left ACC</i>	78	-8, 38, 20	.023
Left PCC	535	-2, -26, 26	<.001	Right precuneus	808	12, -52, 28	.004
				<i>Left PCC</i>	178	-2, -30, 34	.034
Group differences in visual networks							
HeM > FtM			HeW > FtM				
<i>Region</i>	<i>size (voxels)</i>	<i>x, y, z local maxima^a</i>	<i>p value</i>	<i>Region</i>	<i>size (voxels)</i>	<i>x, y, z local maxima^a</i>	<i>p value</i>
<i>Right lateral occipital cortex, inferior^b</i>	183	38, -88, -16	.034	Right cuneus + lateral occipital cortex, superior	4324	24, -84, 12	.004
				Right occipital pole	1493	20, -94, -6	.003
				left occipital fusiform, lingual gyrus, and temporal occipital fusiform gyrus	2917	-14, -80, -12	.003
				Left intracalcarine cortex, supracalcarine cortex, occipital pole	4984	-10, -76, 18	.002
FtM > HeW							
<i>Region</i>	<i>size (voxels)</i>	<i>x, y, z local maxima</i>	<i>p value</i>				
L lingual gyrus	152	-6 -72 6	.011				

^alocal maxima provided in MNI coordinates, whereas "Region" indicates the coverage of the significant clusters. A significance value of $p < .017$, family-wise error corrected, was employed to account for the comparisons among three groups. Italics indicate a subsignificant cluster. There were no significant differences between FtM and HeM or HeW for the salience network, and no significant differences between HeM and HeW for DMN, visual networks, or salience network. HeM = heterosexual male control individuals; HeW = heterosexual women control individuals; FtM = Female-to-male individuals with gender dysphoria.

^b overlapping the extrastriate body area