Originalartikel

Androgens Modulate Brain Networks of Empathy in Female-to-Male Transsexuals: An fMRI Study

Zheng Ye[1*], Robert Kopyciok[1*], Bahram Mohammadi[1,2*], Ulrike M. Krämer[1], Claudia Brunnlieb[1], Marcus Heldmann[1], Amir Samii[2] and Thomas F. Münte[1]

[1]Dept. of Neurology, University of Lübeck, Lübeck, Germany
[2]International Neuroscience Institute, Hannover, Germany
*these authors contributed equally

Abstract. Women show higher sensitivity than men to emotional and social cues and are therefore better in showing empathy with others and in deciphering other's intentions and mental states. These sex differences have been linked to hormonal levels. However, it remains unclear how hormones modulate neural mechanisms underlying empathic processes. To assess effects of chronic hormonal treatment, functional magnetic resonance imaging was used in a group of female-to-male transsexuals before and during androgen therapy and a group of female and male controls while they watched pictures portraying emotionally negative or neutral situations (emotional content) involving one or two persons (social relation). Before therapy, the medial superior frontal gyrus and left inferior frontal gyrus showed greater activations for emotional than neutral stimuli. The posterior superior temporal sulcus showed greater activations for emotional vs. neutral stimuli and for social relations relative to pictures of single persons. Long-term androgen administration reduced the pSTS activity in response to emotional stimuli as well as its response to social relation. More importantly, the functional connectivity among frontal, temporal and striatal regions was weakened while the connectivity among limbic regions was strengthened as the androgen level increased during hormone therapy. This pattern of change was similar to the sex difference observed between female vs. male controls. Thus, making a brain more male by the application of androgens not only reduced the activity of a core neural hub but also markedly altered the organization of the brain network supporting emotional and social cognitive processes related to empathy and mentalizing.

Keywords: transsexuals, empathy, frontal cortex, limbic system, fMRI, graph theory

Androgene modulieren empathie-bezogene Hirnantworten in Transsexuellen: eine fMRT Studie

Zusammenfassung. Frauen zeigen gewöhnlich höhere Sensitivität gegenüber emotionalen uns sozialen Hinweisreizen als Männer. Um Effekte chronischer Hormonbehandlung zu erfassen, wandten wir die funktionelle Magnetresonanztomographie in einer Gruppe von Frau-zu-Mann Transsexuellen vor und während einer Androgentherapie an. Vor der Therapie zeigten der mediale Gyrus frontalis superior und der linke Gyrus frontalis inferior größere Aktivierungen für emotionale verglichen mit neutralen Situationen. Der posteriore Sulcus temporalis superior (pSTS) zeigte größere Aktivierungen für emotionale im Vergleich zu neutralen Stimuli und darüber hinaus auch für Bilder, die zwei Personen abbildeten im Vergleich zu Bildern mit einer Person. Die Androgen-Therapie reduzierte diese pSTS Aktivität. Darüber hinaus fand sich die funktionelle Konnektivität zwischen frontalen, temporalen und striatalen Regionen nach Androgentherapie vermindert, während sie zwischen limbischen Regionen zunahm.

Schlüsselwörter: Transsexualität, Empathie, frontaler Kortex, limbisches System, funktionelle MRT, Graph-Theorie

Introduction

Transsexualism is characterized by a strong and persistent identification with another gender, a long-standing discomfort with one's biological sex and/or a sense of incongruity with one's gender role (DSM-IV TR; ICD-10). It is usually accompanied by a pursuit of sex reassignment through surgery and hormonal treatment. For example, female-to-male transsexuals use androgens to induce male body features, such as a low voice, facial and body hair growth, and a more masculine body shape (for a review, see Cohen-Kettenis & Gooren, 1999). The question arises,

whether such treatments will also change other psychological traits, e.g. the ability to react empathically, from a predominantly female to a male pattern.

Empathy refers to the ability to share feelings we observe in others on the basis of emotional or social cues (Decety & Jackson, 2004; Preston & de Waal, 2002). There is strong evidence for sex differences in empathy. Women are generally more sensitive and responsive to emotional and social cues than men. For example, they are more accurate in decoding facial expressions in both adults (Rotter & Rotter, 1988; Thayer & Johnsen, 2000) and children (Proverbio, Matarazzo, Brignone, Del Zotto & Zani, 2007). They are especially reactive when facing pictures portraying humans in negative contexts, which is also reflected in a larger late parietal positivity in the event-related potential (Proverbio, Adorni, Zani & Trestianu, 2009). Using functional neuroimaging, a greater activity in the prefrontal cortex, superior temporal sulcus and amygdala has been observed in women compared to men in empathy settings (Derntl et al., 2010; Hofer et al., 2006; Klein et al., 2003; McClure et al., 2004).

Sex differences in empathy have been linked to the level of prenatal androgens (Hines, 2008; Knickmeyer, Baron-Cohen, Raggatt, Taylor & Hackett, 2006). Due to ethical constraints, information about the early influence of androgens on the human brain and behavior has been mostly obtained in girls with congenital adrenal hyperplasia (CAH). CAH girls are exposed to unusually high levels of androgens in prenatal and neonatal periods because of a deficiency in the enzyme needed to produce cortisol and a consequent overproduction of testosterone and other androgens. Compared to unaffected girls, CAH girls showed increased interest in boys' toys (Berenbaum, Duck & Bryk, 2000; Berenbaum & Hines, 1992; Ehrhardt, Epstein & Money, 1968; Nordenström, Servin, Bohlin, Larsson & Wedell, 2002; Slijper, 1984) and greater preferences for boys as playmates (Berenbaum & Snyder, 1995). Moreover, CAH women had decreased openness to sensitive feelings or emotions (Mathews, Fane, Conway, Brook & Hines, 2009) but increased coldness in social relations (Helleday, Edman, Ritzen & Siwers, 1993). The assumption that androgenic hormones alter empathy processing even in adulthood is further supported by behavioral studies with healthy women taking a single dose of testosterone (e.g. 0.5 mg in Van Honk & Schutter, 2007). Testosterone administration induced a reduction in conscious detection of facial threat (Van Honk & Schutter, 2007) as well as a reduction in unconscious mimicry response (Hermans, Putman & Van Honk, 2006).

Recent neuroimaging studies on transsexuals have begun to examine how hormonal treatment influences cognitive processes for which sex differences are known, focusing on three-dimensional mental rotation, a spatial task mastered typically more efficiently by men. Male-to-female transsexuals receiving estrogens showed lower parietal activity than male controls but higher ventral medial prefrontal activity than female controls (Carrillo et al., 2010; Schöning et al., 2010). They also showed larger temporal and parietal activity but less dorsolateral prefrontal activity than male-to-female transsexuals before hormone therapy (Schöning et al., 2010).

Up to now, no neuroimaging study has examined how androgens modulate empathy processing. To assess the effect of chronic hormonal treatment, we used functional magnetic resonance imaging (fMRI) in a group of female-to-male transsexuals before and during hormone therapy (pre- vs. post-test) and a matched group of female and male control participants, with a set of picture stimuli portraying emotionally negative or neutral situations (emotional content) involving one or two persons (social relation). This set has been used in previous neuroimaging studies on empathy and theory of mind (Krämer, Mohammadi, Donamayor, Samii & Münte, 2010; Taubner, Münte, Krämer & Wiswede, 2011). Krämer et al. (2010) have shown that no explicit instruction is necessary to activate regions associated with empathy when participants are confronted with emotional or social stimuli. They found the medial prefrontal cortex, ventral lateral prefrontal cortex and posterior superior temporal sulcus to be associated with emotional and/or social cognitive components of empathy processing. This pattern was similar to other neuroimaging studies of empathy using different types of stimuli (Amodio & Frith, 2006; Derntl et al., 2010; Hynes, Baird & Grafton, 2006; Saxe & Wexler, 2005; Shamay-Tsoory & Aharon-Peretz, 2007; Völlm et al., 2006).

In the current study we tested the straightforward hypothesis that androgens can change the neural patterns of empathy processing from a predominant female pattern to a male pattern. This hypothesis was tested with two independent statistical analyses, a standard univariate analysis and a graph-theory-based network analysis. The standard univariate analysis was performed to examine how hormone therapy influences brain activity in response to emotional and social cognitive cues. We expected the activity difference between pre- and post-tests in transsexuals would be similar to that between female and male controls. Secondly, the graph theoretical analysis was performed to examine how hormone therapy alters the organization of the brain network that supports empathic processes. Graph theory has been used to analyze the topological organization of cortical systems in nonhuman primates since the early nineties (Stephan et al., 2000; Young, 1992). Recently it was applied to human anatomical and functional imaging data (Achard & Bullmore, 2007; Bassett et al., 2008; Bassett, Meyer-Lindenberg, Achard, Duke & Bullmore, 2006) as it provides a concise quantification of the extraordinary complexity of interregional connectivity in the human brain (for reviews, see Bullmore & Bassett, 2011; Bullmore & Sporns, 2009). The fundamental assumption of this approach is that the brain has properties of a small-world network which refers to economical systems that are both globally and locally efficient in transferring information (Latora & Marchiori, 2001; Watts & Strogatz, 1998). This assumption has been confirmed by empirical studies on the nervous system of C. elegans, cortical networks in the cat, macaque (Hilgetag, Burns, O'Neill, Scannell & Young, 2000; Sporns,

Chialvo, Kaiser & Hilgetag, 2004; Stephan et al., 2000) and human (Salvador et al., 2005; Stam, 2004). Within the framework of the small-world model, topological measures such as centrality can be used to determine the relative importance of each region in the brain network (i. e., to identify "hubs" and "non-hubs") and to monitor the change of the connectivity pattern of each region during hormonal treatment (e. g. whether a region gained or lost connections with other regions). We expected the between-test difference in transsexuals would be similar to the between-sex difference in controls.

Methods

All procedures had been cleared by the ethical review board of the University of Magdeburg, the affiliation of the senior author at the time of the experiment. Experiments were carried out according to the declaration of Helsinki.

Transsexuals and hormonal treatment

Eight female-to-male transsexuals participated in this study. Each transsexual participant was scanned twice, once before (pre-test) and once during hormonal treatment (post-test; about 12 months after the hormone therapy began). Upon entering the study, they were between 19 and 38 years old (mean age 28.5 years). Seven were right-handed and one was left-handed. All of them had normal or corrected-to-normal vision. Three additional transsexuals were entered to the study but could not be analyzed as they did not participate in the post-test. Results of the pre-test of all 11 transsexuals were essentially similar to the results of 8 transsexuals.

The hormone administration routes and schedules were heterogeneous, as defined by the attending endocrinologist. Three transsexuals received testosterone undecanoate 1000 mg depot injections every 3–4 months (Nebido®), two transsexuals received testosterone enantat 250 mg (Testoviron®) every 2 to 4 weeks and four transsexuals received daily transcutaneous applications of 50 mg testosterone in gel (Testogel®). At the end of the study, all transsexuals had testosterone levels in the range of normal males. The hormonal treatment caused upper body muscle development. The circumference of the biceps muscle of the right arm was 32.4 cm (SD = 4.8 cm) at the pre-test and reached 34.4 cm (4.4 cm) in maximum during hormone therapy.

Healthy controls

Thirty-one women (mean age 24.3, age range 20 to 34 years) and thirty men (mean age 27.1, age range 20 to 39 years old) participated in this study as controls. All of them were right-handed (except one woman) and had normal or corrected-to-normal vision. None of them had any psychiatric or neurological disorder. The results of 16 participants (10 women) have been previously reported in Krämer et al. (2010).

Stimuli and task

Both transsexual and control participants were scanned by using the paradigm of Krämer et al. (2010). Stimuli were black-and-white drawings (see Figure 1) depicting emotionally negative social situations involving two persons (Emot-Two; e. g. two persons standing at a grave), emotionally neutral social situations involving two persons (Neut-Two; e. g. two construction workers working side-by-side), emotionally negative situations involving one person (Emot-One; e. g. a man falling from a sailing boat), emotionally neutral situations involving one person (Neut-One; e. g. an artist painting a picture), or objects (Obj; e. g. a notebook lying on a desk). All depicted emotions were negative and included anger, sadness, pain and anxiety. To avoid processes related to the recognition of emotional facial expressions, faces were deliberately left void of any features (eyes, nose or mouth)[1]. Each condition had 24 pictures.

Participants were asked to watch the pictures carefully during three runs and to complete a recognition test after each run. Each run comprised 40 pictures (8 per condition). These pictures were presented in a pseudo-randomized order to make sure no more than two successive pictures were from the same condition. Each picture was presented for 6 s and followed by a fixation of 10 s. Each test comprised 10 pictures presented in the previous run (2 per condition). Half of the pictures were presented in the same orientation as before, whereas the other half was mirrored. Participants had to indicate whether a particular picture was mirrored or not by pressing the appropriate of two buttons. Functional images were acquired during the presentation of the pictures but not during test phases.

Participants were also asked to fill out a German version of the Interpersonal Reactivity Index (IRI, see Davis, 1983a, 1983b) at pre- and post-tests. This questionnaire comprises four 7-item subscales: Perspective Taking, Fantasy, Empathic Concern and Personal Distress. The Perspective Taking subscale is related to social competence, higher self-esteem and interpersonal competence, but not to affective empathy. Scores on this subscale have been shown to predict the ability to match target persons with their self-descriptions (Bernstein & Davis, 1982). The Fantasy subscale taps the tendency to get deeply involved in the world of books, movies and plays and is correlated with verbal measures and intellectual abilities. The Empathic Concern subscale reflects a person's tendency to have feelings of sympathy and concern for others. Scores on this subscale correlated with persons' emotional reactions after

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[1] The stimuli were inspired by similar style line drawings of the Adult Attachment Projective (AAP) that is used as a psychodiagnostic tool to assess attachment behavior (George & West, 2001).
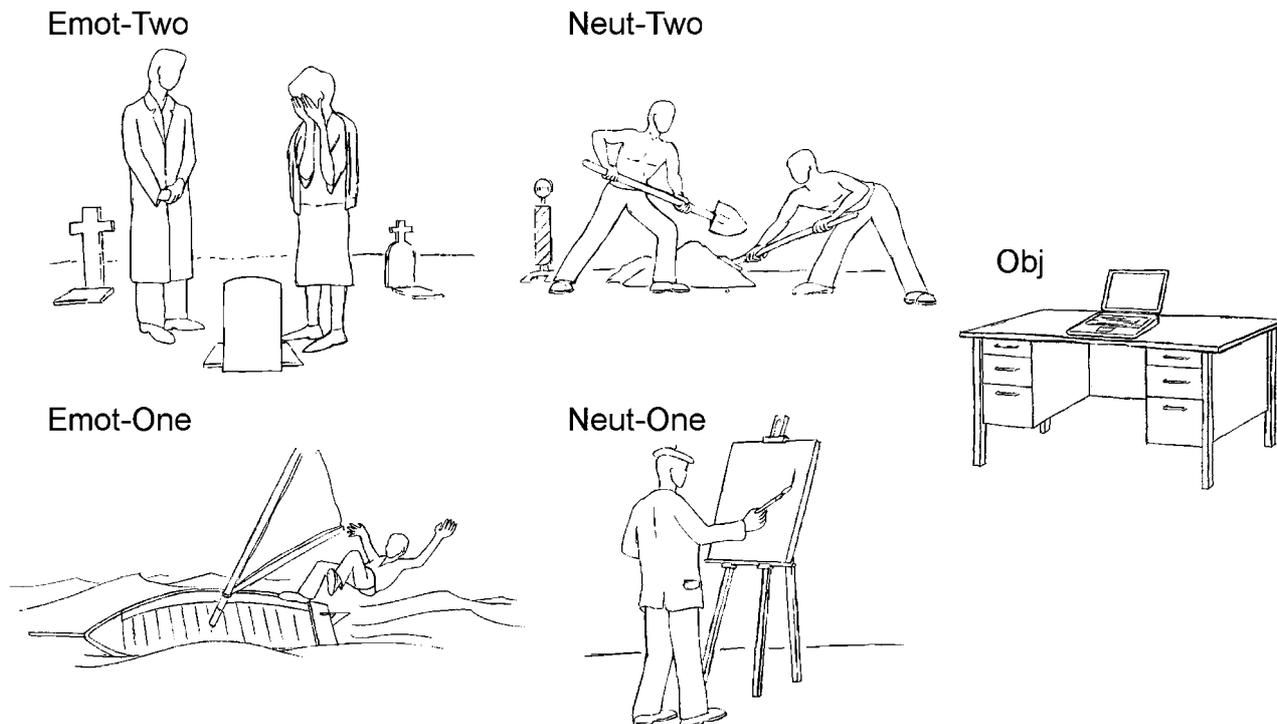
Figure 1. Example black-and-white drawings depicting emotionally negative social situation involving two persons (Emot-Two), emotionally neutral social situation involving two persons (Neut-Two), emotionally negative social situation involving one person (Emot-One), emotionally neutral social situation involving one person (Neut-One), or objects (Obj).

exposure to a young woman in distress and the likelihood of subsequent helping behavior (Davis, 1983a, 1983b). The Personal Distress subscale measures the tendency to experience distress and anxiety in overwhelming social situations such as emergencies.

Data acquisition

Data of transsexuals and controls were all collected on a 3-Tesla Siemens scanner with identical parameters although in different sessions. Functional images were acquired using a Gradient-Echo-EPI-sequence, with 2000-ms time repetition, 30-ms time echo, and 80° flip angle, in three functional runs. Each functional image consisted of 32 transversal slices, with 64*64 matrix, 192 mm*192 mm field of view (FOV), 3-mm thickness, 0.75-mm gap, and 3 mm*3 mm in-plane resolution. Structural images were acquired using a T1-weighted MPRAGE sequence. Each structural image consisted of 192 sagittal slices, with 256*256 matrix, 256 mm*256 mm FOV, and 1 mm isovoxel. The head of participant was fixed during the entire measurement to avoid head movements.

Data analysis

Data were preprocessed with SPM8 (http://www.fil.ion.u-cl.ac.uk/spm). The first four volumes of each functional run were discarded due to equilibration effects. Functional images were first phase-shifted with reference to the middle slice in time to correct differences in slice acquisition time. They were then realigned with a least squares approach and a rigid body spatial transformation to remove movement artifacts. Realigned images were normalized to the EPI-derived MNI template (ICBM152, Montreal Neurological Institute) and resampled to $2 * 2 * 2$ mm^3 voxel size. Normalized images were smoothed with a Gaussian kernel of 8-mm full-width half-maximum and filtered with a high-pass filter of 128 seconds. Two independent analyses were conducted: the standard univariate analysis and the network analysis.

Standard univariate analysis

The standard univariate analysis was performed to examine whether and how brain activity in response to emotional content (Emot vs. Neut) and/or social relation (Two vs. One) was modulated during hormonal treatment in transsexuals (pre- vs. post-test) and/or different between sexes in controls (female vs. male). This analysis was implemented with SPM8 on the basis of a general linear model (GLM) by using one covariate to model hemodynamic responses of all trials of a

condition. Five events were specified and time-locked to their onsets: Emot-Two, Neut-Two, Emot-One, Neut-One and Obj. Estimated movement parameters (six per image: x, y, z, pitch, roll and yaw) were included in the model to minimize signal-correlated motion effect. Classical parameter estimation was applied with a one-lag autoregressive model (AR(1)) to whiten temporal noise in the fMRI time courses in order to reduce the number of false-positive voxels (Smith, Singh & Balsters, 2007). For each participant, contrast maps were calculated for Emot > Neut, and Two > One. The contrast maps were first entered into one-sample t-tests at group level (random effect) to show the effects in each test/sex. Then they were entered into paired-sample t-tests (pre- vs. post-test in transsexuals) or two-sample t-tests (female vs. male controls) to show the interaction between emotional content and test/sex (Emotion x Test/Sex) as well as the interaction between social relation and test/sex (Cognition x Test/Sex). The results of controls were considered at $p < 0.05$ (FDR-corrected) with a minimum cluster size of 20 voxels. We used a relative lenient threshold for transsexuals, $p < 0.005$ (uncorrected) with a minimum cluster size of 20 voxels, in order not to overlook any effect of this small sample (8 subjects). The results were visualized with MRIcron (http://www.cabiatl.com/mricro). We additionally performed an analysis to examine the cognitive processes specific for person vs. object perception. However, these will not be reported here.

Network analysis

The graph-theory-based network analysis was performed to examine whether and how brain networks of empathic processes were modulated during hormonal treatment in transsexuals (pre- vs. post-test) and/or different between sexes in controls (female vs. male). We first constructed a matrix of functional connectivity measures between all possible pairs of regions in the brain with the "beta series correlation" method proposed by Rissman, Gazzaley and D'Esposito (2004). This method was implemented on the basis of a GLM by using separate covariates to model hemodynamic responses of each single trial in each condition. Similar to the univariate model, five types of events were specified and time-locked to their onsets. Estimated movement parameters were included and classical parameter estimation was applied with the AR(1). We segmented each cerebral hemisphere into 45 anatomical regions according to the Automated Anatomical Labeling (AAL, Tzourio-Mazoyer et al., 2002) with the WFU PickAtlas (http://fmri.wfubmc.edu/software/PickAtlas). Beta series of all experimental conditions (except the Obj) were extracted from and averaged within each region. For each participant, correlation coefficients were computed for each possible pair of regions to generate a 90×90 symmetric correlation matrix R, in which $R(i,j)$ represented the functional connectivity between region i and j, where $i \neq j$.

For each correlation matrix R, a binary adjacency matrix A can be derived by applying a threshold value t. $A(i,j)$

is zero if $R(i,j) < t$ and unity if $R(i,j) \geq t$. The adjacency matrix A can further be represented as an undirected graph G, in which a node indicates a region and an edge drawn between two nodes indicates a connection between two regions. For a given graph G, the connection density or topological cost k can be simply defined as the number of edges in G divided by the maximum possible number of edges (e.g. 4005). Obviously the choice of threshold value has a major effect on the topology of G. When $t = 1$, no element of the correlation matrix R can pass the threshold therefore $k = 0$. When $t = 0$, most elements of R will pass the threshold therefore $k \sim 1$ (in this study about 1 % of elements in R was negative). Instead of using one arbitrary value we systematically thresholded R with a range of costs. For each condition, topological measures of the resulting graphs were represented as functions of the cost k. The difference between tests/sexes was examined while the number of edges was exactly the same for each participant (for similar approaches, see Achard & Bullmore, 2007; Bassett et al., 2008).

We investigated topological properties of brain networks with the Brain Connectivity Toolbox (BCT, Rubinov & Sporns, 2010). We focused on regional properties such as centrality that determines the relative importance of a region within the brain network. In order to monitor the change of centrality during hormonal treatment in transsexuals and the difference between sexes in controls, we first established the range of costs that yielded small-world graphs. We then averaged the regional metrics over this small-world regime and conducted statistical tests.

As defined by Watts and Strogatz (1998), a small-world network is a connected graph G with large clustering coefficient but small characteristic path length. The characteristic path length measures the global connectivity of the whole-brain network. Let $L(i,j)$ denote the number of edges in the shortest path between nodes i and j (i.e. distance). If nodes i and j are immediately connected (i.e. neighbors), $L(i,j) = 1$. Then $L(G)$ is the average of $L(i,j)$ over all possible pairs of nodes in G. The clustering coefficient measures the local connectivity of a given region. Suppose G_i is the graph of node i's neighbors and edges between them (i.e. subgraph). Let $C(i)$ denote the number of edges that actually exist divided by the number of edges that could possible exist in Gi. If node i has no neighbor or only one neighbor, $C(i) = 0$. Then the clustering coefficient for the whole network $C(G)$ is the average of $C(i)$ over all nodes in G. The small-worldness of G can be quantified by the scalar σ (Humphries et al., 2006).

$$\sigma = \frac{C(G)/C(R)}{L(G)/L(R)}$$

where $C(R)$ is the clustering coefficient of a comparable random graph and $L(R)$ is the characteristic path length of the random graph. In order to estimate the small-worldness of the whole-brain network at the cost $k = 0.01, 0.02, \ldots,$

0.99, we sampled 100 random graphs with the same number of nodes and edges at each cost. L(R) and C(R) were calculated for each random graph and averaged cross random graphs with the same cost. The upper limit of the small-world regime was defined as the highest cost at which the minimum σ (among all participants) was larger than 1. When σ = 1, the brain network is indistinguishable from a random graph. The lower limit of the small-world regime was defined as the lowest cost at which the minimum clustering coefficient (among all regions) was larger than zero.

There are four measures of centrality that are widely used in network analysis: degree centrality, betweenness centrality, closeness centrality and eigenvector centrality. Degree centrality is the number of edges of a given node. Nodes that have many neighbors show higher degrees. Betweenness centrality is the fraction of all shortest paths that contain a given node. Nodes that occur on many shortest paths between other nodes show higher betweenness. Closeness centrality is the reciprocal of the sum of distances between a given node and all other nodes. Nodes that have short distances to other nodes show higher closeness. Eigenvector centrality is like a recursive version of degree centrality. It starts by assigning centrality score of 1 to all nodes. It re-computes scores of each node as weighted sum of centralities of all neighbors of a given node. It normalizes the scores by dividing each value with the largest value. The last two steps are repeated until values stop changing. We used centrality measures to identify regional hubs of the network. A region was identified as hub, if its value for one of the four measures was at least 2 SDs greater than the mean across all regions in the network. We also used those measures to monitor the change of connectivity pattern of each region.

Results

Behavioral results and questionnaire data

Behavioral and questionnaire results of part of the controls have been reported in Krämer et al. (2010). Here we focus on the data of transsexuals. Transsexual participants performed equally well on the recognition test at the pre- and post-tests (90.8 % vs. 88.9 % correct, t (7) = -1.07, p = 0.32).

With respect to the Interpersonal Reactivity Index, at the pre-test, mean scores were 25.6 (SD = 4.6) on the Perspective Taking subscale, 23.8 (5.4) on the Fantasy subscale, 26.8 (SD = 2.7) on the Empathic Concern subscale, and 17.8 (4.2) on the Personal Distress subscale. At the post-test, mean scores were 25.8 (4.3) on the Perspective Taking subscale, 24.0 (5.6) on the Fantasy subscale, 25.4 (SD = 2.8) on the Empathic Concern subscale, and 16.6 (5.1) on the Personal Distress subscale. The IRI scores were not changed during hormonal treatment (paired-sample t tests: ps > 0.20) or correlated with the fMRI effects of emotional content or social relation (see below).

Brain activity

We first performed the standard univariate analysis to examine whether and how brain activity in response to emotional content (Emot vs. Neut) and/or social relation (Two vs. One) was modulated during hormonal treatment in transsexuals (pre- vs. post-test) and/or different between sexes in controls (female vs. male). Figure 2 and Table 1 show the interaction between emotional content and test/sex (Emotion x Test/Sex) and the interaction between social relation and test/sex (Cognition x Test/Sex). For Emot vs. Neut, the medial superior frontal gyrus (mSFG), left inferior frontal gyrus (LIFG) and posterior superior temporal sulcus (pSTS) showed greater activations at the pre-test in transsexuals and in female controls. In controls, the effect of emotional content over the mSFG and IFG in men was weaker than that in women, as revealed by the Emotion x Sex interaction. In transsexuals, the Emotion x Test interaction was observed in the pSTS, suggesting the effect was weaker at the post- than pre-test. The effect of emotional content over the mSFG (pre-test: maximum t = 7.46, cluster = 705 voxels; post-test: maximum t = 5.21, cluster = 130 voxels) and LIFG (pre-test: maximum t = 6.17, cluster = 117 voxels; post-test: maximum t = 5.77, cluster = 99 voxels) also decreased during hormonal treatment, but the difference between tests did not reach significance (Fig. 2 A). For Two vs. One, the pSTS showed greater activation at the pre-test in transsexuals and in female controls. In transsexuals, the effect of social relation over the pSTS decreased during hormonal treatment, as revealed by the Cognition x Test interaction. However, no comparable difference was obtained in controls (Fig. 2 B). To summarize, the chronic administration of androgenic hormones reduced the STS activity in response to emotional and social stimuli.

Brain networks

We then performed the graph theoretical analysis to examine whether and how brain networks of empathic processes were modulated during hormonal treatment in transsexuals (pre- vs. post-test) and/or different between sexes in controls (female vs. male). To conduct this network analysis, we first established the range of costs in which brain graphs exhibit typical small-world properties, i.e. a balance between local interregional interactions (high clustering coefficient) and long-distant projections (short characteristic path length). Figure S3 shows the small-worldness scalar σ and the minimum clustering coefficient at each cost. The upper limit of the small-world regime was defined by the highest cost at which the minimum σ was larger than 1 (transsexuals: k = 0.26; controls: k = 0.23). The lower limit of the small-world regime was defined by the lower cost at which the minimum clustering coefficient was larger than zero (transsexuals: k = 0.12; controls: k = 0.05). Thus, we defined the range 0.12 ≤ k ≤ 0.23 as the small-world regime of all participants. The subsequent analysis was carried out in this regime. Figure 3 A show
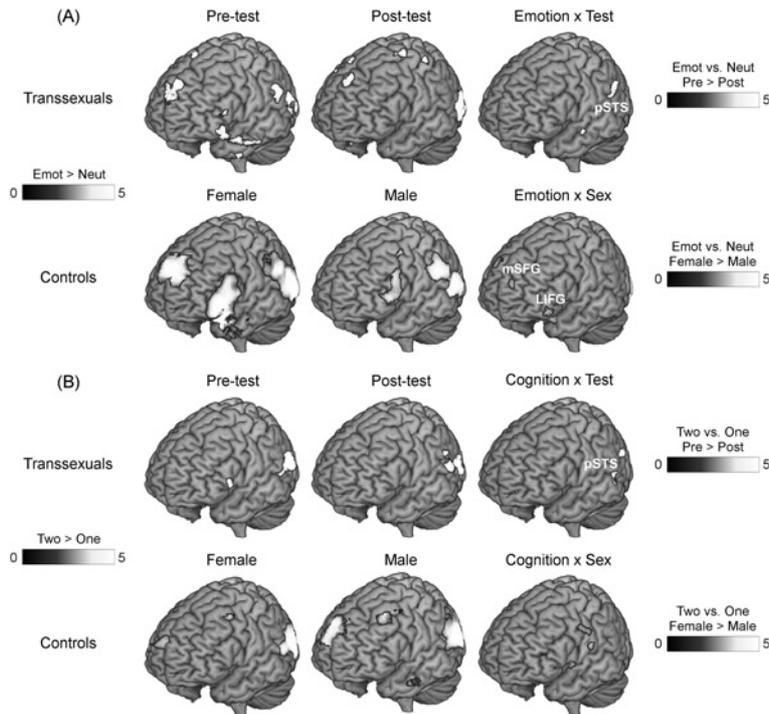
Figure 2. Brain activity in response to (A) emotional content and (B) social relation in female-to-male transsexuals (pre- vs. post-test) and in controls (female vs. male). mSFG, medial superior frontal gyrus; LIFG, left inferior frontal gyrus; pSTS, posterior superior temporal sulcus. Color scales indicate t values.

brain graphs averaged in this small-world regime for transsexuals at the pre- and post-tests as well as for female and male controls. Each circle indicates a region located in the MNI space. Each edge indicates a connection between a pair of regions. The size of a particular circle corresponds to the number of connections that the corresponding region has.

For each brain graph, hubs were identified as regions that had centrality values at least 2SD greater than the mean across all 90 regions. Table 2 shows regional hubs and their numbers of SDs away from the mean. Here we focused on hubs identified by degree and betweenness centrality because neither closeness nor eigenvector centrality successfully identified any hub. Regions such as the bilateral middle cingulate cortex (MCC, left: 6 out of 8; right: 4 out of 8), left middle temporal gyrus (MTG, 7 out of 8) and right superior temporal gyrus (STG, 4 out of 8) were identified as hubs in both transsexuals and controls. We additionally identified hubs for transsexuals and controls in separate small-world regimes (transsexuals: $0.12 \leq k \leq 0.26$; controls: $0.05 \leq k \leq 0.23$). The results were essentially the same (see Tab. S3).

The centrality of regional hubs did not significantly change from pre- to post-test in transsexuals or differ between sexes in controls (see Fig. 2 B; see Tab. S4 for details). On the other hand, there were regional non-hubs of which centrality significantly changed during hormonal treatment in transsexuals and/or differed between female and male controls (corrected with bootstrap resampling 1000 time; see Tab. 3 and Fig. 3 B). Among these regions, four showed similar patterns in transsexuals and controls. The left inferior frontal gyrus (LIFG) and left medial superior frontal gyrus (LmSFG) showed larger centrality, while the left

amygdala (LAmy) and left hippocampus (LHC) showed smaller centrality for pre- vs. post-test and for females vs. males.

As degree centrality specifically measures the number of connections a region has, the change of degree implies that the region gains or loses connections with other regions. To find out which connections a particular region gained or lost during hormonal treatment, we computed the mean brain graph in the small-world regime for each participant. The mean brain graph represented the strength of connectivity between regions. If region i and j had been connected since the lowest cost, the connectivity between them is 1. If region i and j were not connected even at the highest cost, the connectivity between them is 0. We selected four regions-of-interest (ROIs), LIFG, LmSFG, LAmy and LHC, to examine how connectivity between frontal/limbic regions and other regions changed during hormonal treatment in transsexuals and/or differed between female and male controls. We additionally tested the left putamen (LPut) since the striatum usually plays an important role in circuits connecting cortical and subcortical areas. Figure 3 C and Table 4–5 show connections which were strengthened or weakened in transsexuals and controls. The common pattern was plotted separately. The connections of LIFG-left insula (LIns), LIFG-right precentral cortex (RPreC), LmSFG-left inferior temporal gyrus (LIT) and LPut-LSMA were stronger for pre- than post-test and for women than men. By contrast, the connections of LAmy-RAmy, LAmy-RPHC and LHC-RAmy were stronger for post- than pre-test and for men than women. In other words, women and transsexuals before hormonal treatment showed stronger connectivity among frontal, temporal and striatal structures, while men and

Table 1
*Interaction between Emotion/Cognition and Test/Sex in brain activity*

Region	BA	H	x	y	z	t	Number of voxels
Transsexuals: Emot vs. Neut in pre- vs. post-test							
Superior temporal sulcus (posterior)	40/22	L	–56	–48	34	6.37	207
Superior temporal sulcus (middle)	22	L	–52	–26	2	4.84	23
Superior temporal sulcus (anterior)	20	L	–54	–8	–14	5.40	27
Middle frontal gyrus	9	R	22	26	40	9.99	28
Inferior parietal lobule	40	R	54	–42	40	5.08	126
Precuneus		R	14	–54	38	4.22	21
Controls: Emot vs. Neut in females vs. males							
Medial superior frontal gyrus	10	L	–16	60	18	3.20	67
		R	18	56	38	3.10	42
Inferior frontal gyrus	47	L	–50	38	–10	3.50	32
	44	R	60	16	6	3.69	45
Precentral cortex	6	R	60	–4	44	3.60	39
Superior temporal pole	38	L	–38	24	–22	3.21	72
		R	42	22	–24	3.26	125
Middle occipital cortex	18/19	L	–32	–96	10	4.55	687
		R	34	–94	10	4.40	632
Lingual gyrus	17	L	–4	–64	6	2.94	21
	19	R	14	–52	–8	3.04	54
Putamen		R	24	0	12	3.30	25
Transsexuals: Two vs. One in pre- vs. post-test							
Superior temporal sulcus (posterior)	39	L	–58	–64	28	7.29	59
Superior temporal sulcus (middle)	22/21	L	–66	–42	8	6.41	125
Superior frontal gyrus	9	R	22	32	48	5.12	45
Middle frontal gyrus	8	R	30	22	48	5.39	133
Controls: Two vs. One in females vs. males							
Inferior frontal gyrus	45	L	–40	36	8	3.54	144
Postcentral cortex	43	L	–64	–12	20	3.54	23
Superior temporal pole	38	L	–56	6	0	2.98	32
Cuneus	18	L	–4	–88	28	3.56	81
Putamen		L	–28	8	12	3.45	49

BA, Brodmann Area; H, hemisphere; coordinates in MNI; t, statistic values of peaks; L, left; R, right.

transsexuals after hormonal treatment showed stronger connectivity within the limbic system. Interestingly, the LPut connectivity with the bilateral amygdala was stronger for men than women but not for transsexuals after than before hormonal treatment. This explains why the LPut showed a significant decrease of degree centrality in transsexuals but not in controls.

Discussion

In this study we examined how androgens given to female-to-male transsexuals modulate the empathy processing. We first examined the influence of hormone therapy on brain responses to stimuli varying in their emotional content and in their content of social relations. Consistent with Krämer et al. (2010), we observed increased activations in the medial superior frontal gyrus (mSFG), left inferior frontal gyrus (LIFG) and posterior superior temporal sulcus (pSTS) for emotionally negative compared to emotionally
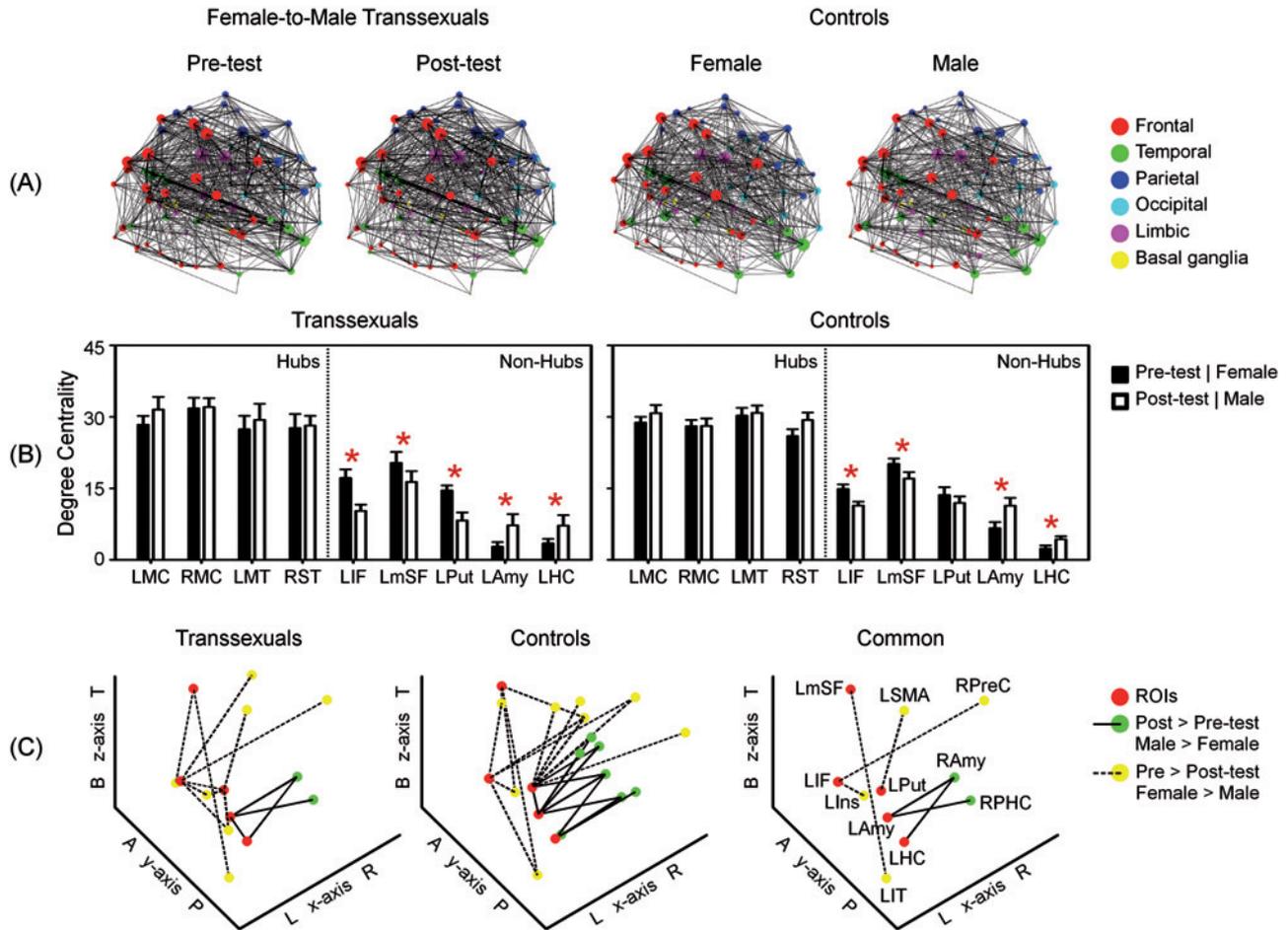
Figure 3. (A) Brain graphs averaged in the small-world regime for transsexuals at the pre- and post-tests as well as for female and male controls. Each circle indicates a region located in the MNI space. Each edge indicates a connection between a pair of regions. The larger a circle is more connections the corresponding region has. (B) Values of degree centrality of regional hubs and non-hubs. Red asterisks indicate significant changes, $p < 0.05$. L, left; R, right; MC, middle cingulate cortex; MT, middle temporal gyrus; ST, superior temporal gyrus; IF, inferior frontal gyrus; mSF, medial superior frontal gyrus; Put, putamen; Amy, amygdala; HC, hippocampus. (C) Connections significantly strengthened (green circles and solid lines) or weakened (yellow circles and dashed line) in transsexuals, controls or both. L, left; R, right; A, anterior; P, posterior; B, bottom; T, top; SMA, supplementary motor area; PreC, precentral cortex; Ins, insula; IT, inferior temporal gyrus; PHC, parahippocampus.

neutral pictures, and increased pSTS activation for pictures portraying two persons in social relations relative to single persons in transsexuals before hormonal treatment. Chronic hormonal treatment reduced the pSTS response to both emotional and social cognitive aspects of the stimuli. However, this pattern was not consistent with the sex differences observed in controls, which were mainly located in the prefrontal cortex including the mSFG and LIFG. Unlike Krämer et al. (2010) we did not obtain a correlation between brain activity and IRI subscales in transsexuals, which may be due to the small sample size.

Then we examined how the organization of the brain network supporting empathic processes was modulated and found common patterns in transsexuals and controls. On the one hand, the bilateral middle cingulate cortex, left middle temporal gyrus and right superior temporal gyrus

functioned as regional hubs in both transsexuals and controls. The centrality (relative importance) of these regions did not change during hormone therapy or differ between sexes. Note that, regions along the STS but not the STS itself are labeled in the AAL. The presence of the middle and superior temporal gyri as hubs may actually reflect the crucial role of STS in empathic processes. On the other hand, the connections among frontal, striatal and limbic systems were altered as the androgen level increased. To be specific, the connectivity within the frontal lobe (e.g. between the LIFG and left insular cortex), the connectivity between frontal and temporal lobes (e.g. between the left mSFG and left inferior temporal gyrus), and the connectivity between frontal and striatal structures (e.g. between the left supplementary motor area and left putamen) were stronger in transsexuals before than after hormonal treat-

Table 2
Regional hubs in the small-world regime for transsexuals and controls

Region	H	Degree Centrality				Betweenness Centrality			
		Pre	Post	Female	Male	Pre	Post	Female	Male
MCC	L		2.44		2.39	2.10	2.35	2.00	2.87
	R	2.43	2.52			3.27	2.59		
STG	R				2.16	2.03	2.11		2.32
MTG	L		2.11	2.10	2.40	2.11	3.98	3.05	3.07
	R			2.07				2.46	
SFG	R							2.18	
ITG	R							2.14	
STP	L						2.61		
SMA	L		2.12						
Precuneus	R					2.14			
Thalamus	L					2.27			

Regional hubs were identified in the range $0.12 \le k \le 0.23$ for both transsexuals and controls. Values indicate the numbers of SDs away from the mean across all 90 regions. H, hemisphere; L, left; R, right; MCC, middle cingulate cortex; STG, superior temporal gyrus; MTG, middle temporal gyrus; SFG, superior frontal gyrus; ITG, inferior temporal gyrus; STP, superior temporal pole; SMA, supplementary motor area.

Table 3
Regional non-hubs showing significant changes in centrality.

Region	H	Degree Centrality		Betweenness Centrality	
		Pre vs. Post	Female vs. Male	Pre vs. Post	Female vs. Male
IFG	L	–3.08**	–2.79**	–2.86**	
	R				2.66**
mSFG	L	–2.38*	–1.76*		–2.52**
	R	–1.85			
Precentral	L		–2.91**		
IPL	L		–2.40*		
Putamen	L	–3.17**			–2.64*
Calcarine	L	2.38*			
Lingual	L	1.96*			
Amygdala	L	1.77	–2.29*		
	R		2.76**	1.79	
HC	L	1.76	2.09*		
PHC	L		4.65**		1.99*
	R		5.03**		

Values indicate statistical values of the paired-sample t-test in transsexuals or the two-sample t test in controls (corrected with bootstrap resampling 1000 times). Negative values: pre-test > post-test, or female > male; positive values: post-test > pre-test, or male > female. Asterisks indicate significances: , $p < 0.08$; *, $p < 0.05$; **, $p < 0.01$. H, hemisphere; L, left; R, right; IFG, inferior frontal gyrus; mSFG, medial superior frontal gyrus; IPL, inferior parietal lobule; HC, hippocampus; PHC, parahippocampus.

ment, as well as in female than male controls. By contrary, the connectivity within the limbic system (e. g. between the amygdala, hippocampus and parahippocampus) was stronger in transsexuals after than before hormonal treatment, and in male than female controls. Therefore we suggest that long-distant projections connecting the frontal cortex, temporal lobe and striatum may be more important when the androgen level is relatively low (i. e. in the range of normal women), whereas limbic circuits may be more crucial when the androgen level is relatively high (i. e. in the range normal men). This hypothesis needs to be tested in further studies.

Frontal and temporal structures such as the mSFG, IFG and STS have been linked to empathic processes (Schulte-

Table 4
Weakened interactions among frontal regions, striatum and other brain areas

Region	H	L IFGoper		L mSFG		L Putamen	
		Pre vs. Post	Female vs. Male	Pre vs. Post	Female vs. Male	Pre vs. Post	Female vs. Male
SFG	L		–2.55*		–2.54*		
	R	–2.15					
SMA	L					–2.40*	–1.90
	R						–2.39*
PreC	R	–3.85**	–1.95				–2.27*
LIns	L	–2.95*	–2.01*		–2.01*	–2.72*	
IFGoper	L	–	–			–2.79*	
IFGorb	L			–2.46*			
	R		–2.08*		–2.08*		–2.13*
ITG	L		–2.35*	–3.26*	–2.35*		
SMG	L	–2.78*				–2.58*	
	R						–2.07*
Putamen	L	–2.79*				–	–

Values indicate statistical values of the paired-sample t-test in transsexuals or the two-sample t test in controls. Negative values: pre-test > post-test, or female > male; positive values: post-test > pre-test, or male > female. Asterisks indicate significances: , p < 0.08; *, p < 0.05; **, p < 0.01. L, left; R, right; IFG, inferior frontal gyrus (oper, pars opercularis; orb, pars orbitalis); mSFG, medial superior frontal gyrus; SMA, supplementary motor area; PreC, precentral cortex; Ins, insula; ITG, inferior temporal gyrus; SMG, supramarginal gyrus; MCC, middle cingulate cortex.

Table 5
Strengthened interactions among limbic regions, striatum and other brain areas

Region	H	L Amygdala		L HC		L Putamen	
		Pre vs. Post	Female vs. Male	Pre vs. Post	Female vs. Male	Pre vs. Post	Female vs. Male
Putamen	L		2.09*			–	–
	R		2.71**				2.02*
MCC	L		3.17**				
	R		2.11*				–2.05*
Amygdala	L	–	–	3.03*			2.09*
	R	2.54*	3.19**	3.00*	2.92**		2.96**
HC	L	3.03*		–			
	R				3.02**		
PHC	L				2.11*		
	R	2.39*	2.55*		2.85**		

Values indicate statistical values of the paired-sample t-test in transsexuals or the two-sample t test in controls. Negative values: pre-test > post-test, or female > male; positive values: post-test > pre-test, or male > female. Asterisks indicate significances: , p < 0.08; *, p < 0.05; **, p < 0.01. L, left; R, right; MCC, middle cingulate cortex; HC, hippocampus; PHC, parahippocampus.

Rüther, Markowitsch, Fink & Piefke, 2007; Shamay-Tsoory, Aharon-Peretz & Perry, 2009) or emotional processing in a more general sense (Phan, Wager, Taylor & Liberzon, 2002). In a recent fMRI study, Schulte-Rüther and colleagues (2008) examined sex differences in the emotional component of empathy processing by using faces with angry or fearful expressions. They asked participants to evaluate the emotional state of a particular face (other-task), to indicate their own emotional responses to that face (self-task, empathy task), or to indicate the gender and age of the face (baseline). Both, women and men showed inc-reased activations in the mSFG, bilateral IFG and bilateral STS in self- and other-tasks relative to baseline. However, only the STS and right IFG showed sex differences in the self-task, with women showing greater activations than men in the right STS and right IFG and men showing greater activations in the left STS. Consistent with these findings, we found that the IFG activity is different between sexes and the STS activity is influenced by androgen levels. The current results further suggest that the STS activity sensitive to social relation is also reduced as a result of androgen treatment. To the extent to which the degree of activation

of the pSTS reflects the person's involvement in the task, this implies that an increased androgen level results in less engagement in the analysis of social relations between two persons.

It is worth noting that neither the mSFG, nor the LIFG or pSTS can be regarded to be specific to empathy processing. They rather reflect processes related to mentalizing in a more general sense. Mentalizing refers to the understanding of others' intentions, beliefs and feelings, in distinction to one's own beliefs and feelings. Increased activations of the mSFG and pSTS have consistently been observed when participants make inferences about others' mental states (Castelli, Happé, Frith & Frith, 2000; den Ouden, Frith, Frith, & Blakemore, 2005; Fletcher et al., 1995; Gallagher et al., 2000; Gobbini, Koralek, Bryan, Montgomery & Haxby, 2007; Hynes et al., 2006; Peelen, Atkinson & Vuilleumier, 2010; Völlm et al., 2006; von dem Hagen et al., 2009; Zaki, Weber, Bolger & Ochsner, 2009) or monitor their own mental states (Gusnard, Akbudak, Shulman & Raichle, 2001) which could be emotional or not. Their co-activation has also been found in a range of situations in which mentalizing is likely to be involved, such as understanding metaphors (Bottini et al., 1994) and ironic expressions (Shibata, Toyomura, Itoh & Abe, 2010; Uchiyama et al., 2006), evaluating the emotional content of spoken and written languages (Beaucousin et al., 2007; Takahashi et al., 2004), looking into the eyes of others (Calder et al., 2002; Kuzmanovic et al., 2009), adopting the visual perspective of a third person (Dumontheil, Küster, Apperly & Blakemore, 2010), observing a pretend play (e. g. a person played a tennis racket as a banjo; Whitehead, Marchant, Craik & Frith, 2009) or being involved in a gamble in which gain or loss of one participant is determined by the behavior of another (Fukui et al., 2006). Gallagher and Frith (2003) proposed that the mSFG supports cognitive processes specific to the attribution of mental states, such as the ability to decouple one's mental state from reality (Gallagher & Frith, 2003). This hypothesis has been criticized by others who suggested that the mSFG rather supports domain-general processes such as the initiation and maintenance of non-automatic cognitive processes (Ferstl & von Cramon, 2002; Zysset, Huber, Ferstl & von Cramon, 2002). The later hypothesis is consistent with findings of executive control studies in which the dorsal mSFG is assumed to be crucial for maintaining internal goals, monitoring conflicts and adjusting cognitive processes in sensory, memory and motor systems (Botvinick, Braver, Barch, Carter & Cohen, 2001; Botvinick, Nystrom, Fissell, Carter & Cohen, 1999; Dosenbach et al., 2007, 2006). With respect to the pSTS, it has been suggested that this area is responsible for integrating information from the dorsal and ventral visual streams which is a necessary prerequisite for the understanding of others' intentions but not the core process of mentalization (Allison, Puce & McCarthy, 2000; Gallagher & Frith, 2003). Such a hypothesis is consistent with recent findings that the STS plays an important role in auditory-visual integration (Chandrasekaran & Ghazanfar, 2009; Noesselt et al., 2007;

Taylor, Moss, Stamatakis & Tyler, 2006). In addition, the LIFG (Broca's area) is usually assumed to support the controlled semantic retrieval and lexical selection during word comprehension and production (Badre & Wagner, 2007; Mestres-Missé, Càmara, Rodriguez-Fornells, Rotte & Münte, 2008; Thompson-Schill, D'Esposito, Aquirre & Farah, 1997; Ye, Mestres-Missé, Rodriguez-Fornells & Münte, 2011), syntactic processes during sentence comprehension (Caplan, 2001; Friederici, 2011) or unification across different linguistic levels during language processing (Hagoort, 2005).

The absence of amygdala activity for emotionally negative vs. neutral pictures in the current study was somewhat unexpected as previous observations found greater amygdala activation when participants imitated others' facial expressions (empathy task; see Carr, Iacoboni, Dubeaut, Mazziotta & Lenzi, 2003) and when people inferred others' emotional states [mentalizing task; see Schnell, Bluschke, Konradt & Walter, 2011). Existing findings regarding sex differences in the amygdala are neither consistent nor well understood. Some neuroimaging studies reported increased amygdala in women compared to men (Derntl et al., 2010; Klein et al., 2003), while others reported the left amygdala to be equally responsive in men and women whereas right amygdala activity was only observed in women (Proverbio et al., 2009). A consideration of neuroanatomical differences reveals yet another pattern. The grey matter density/volume of the left amygdala has consistently been observed to be larger in adolescent and adult males than females (Goldstein et al., 2001; Neufang et al., 2009; Peper et al., 2009). Moreover, the volume of the left amygdala was positively correlated with an individual's testosterone level during brain development, regardless of sex (see Lenroot & Giedd, 2009, for a review; Neufang et al., 2009). The current findings further suggest that the functional connectivity pattern of the left amygdala can also be affected by the androgen level.

Empathy and mentalizing about the intentions and social relations of others is considered to be a more prominent trait of the female sex. The present results show that making a brain more male by the chronic administration of androgens reduces the activity in one of the core hubs of the brain network related to mentalizing and empathy, namely the pSTS. It further suggests that the increased level of androgenic hormones gives rise to stronger connections among limbic structures but weaker connections among frontal, temporal and striatal regions, resulting in an altered brain functional network.

Acknowledgments

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Prof. Dr. med. Thomas F. Münte, MD

Department of Neurology
University of Lübeck
Ratzeburger Allee 160
DE-23538 Lübeck
E-mail: Thomas.muente@neuro.uni-luebeck.de