NeuroImage 138 (2016) 257–265

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Gender transition affects neural correlates of empathy: A resting state functional connectivity study with ultra high-field 7T MR imaging

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ARTICLE INFO

Article history:
Received 14 December 2015
Revised 17 May 2016
Accepted 24 May 2016
Available online 26 May 2016

Keywords:
Gender transition
Transgender
Network based statistics
Resting state functional connectivity
Empathy

ABSTRACT

Sex-steroid hormones have repeatedly been shown to influence empathy, which is in turn reflected in resting state functional connectivity (rsFC). Cross-sex hormone treatment in transgender individuals provides the opportunity to examine changes to rsFC over gender transition. We aimed to investigate whether sex-steroid hormones influence rsFC patterns related to unique aspects of empathy, namely emotion recognition and description as well as emotional contagion. RsFC data was acquired with 7 Tesla magnetic resonance imaging in 24 male-to-female (MtF) and 33 female-to-male (FtM) transgender individuals before treatment, in addition to 33 male- and 44 female controls. Of the transgender participants, 15 MtF and 20 FtM were additionally assessed after 4 weeks and 4 months of treatment. Empathy scores were acquired at the same time-points. MtF differed at baseline from all other groups and assimilated over the course of gender transition in a rsFC network around the supramarginal gyrus, a region central to interpersonal emotion processing. While changes to sex-steroid hormones did not correlate with rsFC in this network, a sex hormone independent association between empathy scores and rsFC was found. Our results underline that 1) MtF transgender persons demonstrate unique rsFC patterns in a network related to empathy and 2) changes within this network over gender transition are likely related to changes in emotion recognition, -description, and -contagion, and are sex-steroid hormone independent.

Introduction

Numerous studies have demonstrated sex differences in the functional organization of the brain. Functional magnetic resonance imaging (fMRI) has allowed for elucidation of sex differences in functional connectivity, for example during utilization of working memory (Hill et al., 2014) and affective regulation (Moriguchi et al., 2014). In addition, sex differences have also consistently been shown in functional connectivity during the resting state (Zuo et al., 2010; Casanova et al., 2012; Wu et al., 2013; Hjelmervik et al., 2014; Scheinost et al., 2015). Individual studies are underlined by a large, multicenter investigation by Biswal et al., which assessed resting state functional connectivity (rsFC) in over 1400 healthy controls and demonstrated a decisive influence of sex, particularly in medial brain regions (Biswal et al., 2010). In transgender individuals, cortical thickness (Manzouri et al., 2015; Luders et al., 2012), gray matter volume (Simon et al., 2013), white matter microstructure (Kranz et al., 2014) as well as structural connectivity (Hahn et al., 2015), and rsFC (Lin et al., 2014) have been shown to differ from controls groups. However, changes to rsFC over the course of gender transition induced by cross-sex hormone treatment have yet to be investigated.

One may assume that the sexual dimorphism in rsFC demonstrated in cis-sexual individuals, i.e. persons that demonstrate concordant gender identity and biological sex, may be related to differences in sex-steroid hormones, as these obviously differ between the sexes. However, research on this topic is limited. A recent study reported an influence of sex-steroid hormones by showing rsFC changes related to the menstrual cycle and oral contraceptive use in the default mode and executive control networks (Petersen et al., 2014). An investigation of 32 scans across the menstrual cycle of a single female subject also demonstrated an influence of sex-steroid hormones on rsFC between the dorsolateral prefrontal cortex, sensorimotor regions, and hippocampal regions, to the rest of the brain (Arelin et al., 2015). Furthermore, it has been suggested that estrogen may modulate rsFC between the amygdala and other brain regions (Engman et al., 2016). In contrast, Hjelmervik et al. found no influence of sex-steroid hormones and

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http://dx.doi.org/10.1016/j.neuroimage.2016.05.060
1053-8119/

argue that observed rsFC differences between males and females are likely to be fundamentally sex rather than hormone related (Hjelmervik et al., 2014). Thus, further studies investigating possible modulation of rsFC by sex-steroid hormones are warranted.

On the other hand, numerous studies have demonstrated that predisposition for empathic function is reflected in rsFC patterns. RsFC within the default mode network assessed before exposure to visual stimuli depicting painful experiences was shown to correlate with the extent of subjects' empathic response (Otti et al., 2010). Another investigation showed increased rsFC in regions often associated with empathic response, more specifically the medial prefrontal cortex and lateral paralimbic regions, in persons exhibiting personality traits with predilection to empathic concern (Adelstein et al., 2011). Similarly, tendency for empathizing, rather than systemizing, was associated with higher rsFC between the medial prefrontal cortex, dorsal anterior cingulate cortex, precuneus, and superior temporal regions (Takeuchi et al., 2014). Accordingly, patients with high functioning autism, which is associated with deficits in empathic processing, also show altered rsFC (Mueller et al., 2013).

Interestingly, behavioral and neural markers of empathy show sex differences (Takeuchi et al., 2014; Baron-Cohen and Wheelwright, 2004) and are influenced by sex hormones (Witte et al., 2010). Estradiol and testosterone have been shown to influence empathic behavior (Bos et al., 2012) and structural changes within regions associated with empathy (Morelli et al., 2014) were influenced by sex-steroid hormone levels (Witte et al., 2010). In fact, it has been suggested that autism, which is related to deficits in empathy, may be associated with excessive brain masculinization (Baron-Cohen et al., 2014).

Therefore, extensive research demonstrates that empathy's neuronal correlates entail specific rsFC patterns. Although behavioral studies on empathy show decisive sex and hormone effects, the extent to which sex-steroid hormones modulate the association between empathy and rsFC is not fully understood.

This novel study employs cross-sex hormone treatment in transgender persons as a model to investigate the relationship between sex-steroid hormones, empathy, and rsFC. In order to differentially assess unique aspects of empathic processing we used the Bermond-Vorst Alexithymia Questionnaire (BVAQ) (Vorst and Bermond, 2001) and Emotional Contagion Scale (ECS) (Doherty, 1997). The BVAQ assesses proneness to alexithymia, which is characterized by deficits in emotion recognition and description (Vorst and Bermond, 2001; Lane et al., 2015), while the ECS assesses emotional contagion (Doherty, 1997), or proneness for transfer of emotion (Bird and Viding, 2014). Emotion recognition and attribution as well as emotional contagion can be understood as components of empathic function (Bird and Viding, 2014).

For rsFC we utilized network based statistics (NBS), which exhibits multiple benefits in comparison to other methods for assessment of functional connectivity. Most importantly, it overcomes seed selection bias and is therefore explorative rather than hypothesis driven (Cole et al., 2010). This study aims to utilize this model to investigate 1) putative unique rsFC patterns in transgender individuals, 2) their changes over gender transition, and 3) to which extent empathy's rsFC correlates are mediated by sex-steroid hormones and hereby reflect behavioral data.

Materials and methods

Subjects

24 male-to-female (MtF, mean age ± SD = 30.25 ± 8.07), 33 female-to-male (FtM, 26.79 ± 6.36), 33 male controls (MC, 27.48 ± 6.78), and 44 female controls (FC, 26.16 ± 6.07) were included in this study. Transgender participants were recruited from the Department of Gynecology and Obstetrics, Unit for Gender Identity Disorder, at the Medical University of Vienna, while controls were recruited via advertisement in the community. All transgender subjects fulfilled the Diagnostic and Statistical Manual of Mental Disorders (4th ed., text rev.; DSM-IV-TR; American Psychiatric Association, 2000) diagnoses of gender identity disorder. Gender identity disorder is characterized by a strong incongruence between a person's experienced or expressed gender and their assigned gender, which persists over more than 6 months and causes clinically significant distress and impairment in daily life (American Psychiatric Association, 2000). All transgender participants were free of current psychiatric comorbidities, which were assessed using the Structured Clinical Interview for DSM-IV Disorders (SCID), though some subjects showed a history of previous depressive symptoms (9 FtM, 3 MtF) and substance abuse (5 FtM, 1 MtF). 3 FtM, 3 FtM, and 1 MtF showed a previous history of eating disorders, anxiety symptoms, and obsessive–compulsive symptoms, respectively. The relatively high rate of psychiatric symptoms is to be expected considering the risk for psychiatric comorbidities in transgender persons (Terada et al., 2012). In control subjects, the SCID was used to exclude all current or past psychiatric disorders. At screening, all participants underwent standard medical examination including a physical examination, routine laboratory testing and electrocardiography as well as a thorough medical history in order to exclude severe internal or neurological illnesses. Urine pregnancy testing was performed in females to exclude pregnancy and breastfeeding females were excluded from the study. Drug-urine tests were performed to exclude current substance abuse. Transgender subjects with current substance abuse and controls with any history of substance abuse were excluded. Additional exclusion criteria included all contraindications for magnetic resonance imaging (MRI) measurement including implants, pacemakers, and claustrophobia. All subjects provided written informed consent and received financial reimbursement for participation. This study was approved by the Ethics Committee of the Medical University of Vienna and was performed according to the Declaration of Helsinki.

Cross-sex hormone treatment

All transgender participants were seeking cross-sex hormone treatment, to which they were naïve at the screening visit. Medical care related to cross-sex hormone treatment was provided by, and followed protocols routinely implemented at, the Department of Obstetrics and Gynecology, Unit for Gender Identity Disorder, Medical University of Vienna. In Austria, the recommendations for gender transition are based on the Standards of Care for the Health of Transsexual, Transgender, and Gender Nonconforming People, 7th Version, set forth by The World Professional Association for Transgender Health, Atlanta, Georgia, 2011. For FtM, cross-sex hormone treatment consisted of 1000 mg testosterone undecanoate administered every 8–12 weeks (Nebido 250 mg/mL 4 mL vial, intramuscular; Bayer, Vienna, Austria, 17 subjects) or 50 mg testosterone daily (Testogel 50 mg/5 g bag, transdermal; Bayer, Vienna, Austria, 3 subjects). One case received 10–15 mg lynestrenol daily (Orgametril 5 mg tablet, oral; Organon, Oss, The Netherlands) to support cessation of menstruation in addition to testosterone treatment. In MtF, 25 to 50 mg cyproterone acetate daily (Androcur 50 mg tablet, oral; Bayer, Vienna, Austria, 8 subjects) or 4.12 mg triptorelin acetate every 4–6 weeks (Decapeptyl 172 mg powder for suspension for injection, subcutaneous or intramuscular; Ferring Arzneimittel, Vienna, Austria, 5 subjects) was given, while two MtF did not receive antiandrogen or GnRH analog treatment. MtF were also prescribed either 75–100 μg estradiol daily (via a transdermal therapeutic system applied twice a week; Estradot, Novartis, Vienna, Austria or Estramon, Hexal, Vienna, Austria, 2 subjects) or 4 mg estradiol hemihydrate daily (Estrofem 2 mg tablet, oral; Novo Nordisk, Vienna, Austria, 6 subjects). Seven MtF subjects received 0.75 to 1.5 mg estradiol hemihydrate daily (Estrogel 0.75 mg/pump, transdermal; Meda, Vienna, Austria). Five MtF additionally received 2.5 mg finasterid every 1–2 days (5 mg tablet, oral; Ratiopharm, Vienna, Austria) in

order to prevent extensive hair loss. None of the subjects underwent sex reassignment surgery before or during our study.

MRI scanning

All participants underwent baseline resting state fMRI (MRI1) before start of cross-sex hormone treatment. 15 MtF and 20 FtM were further assessed with fMRI 4 weeks (MRI2) and 4 months (MRI3) after the beginning of cross-sex hormone treatment. MRI was performed at the Medical University of Vienna on a 7 T scanner (Siemens Magnetom, Erlangen, Germany) using a 32-channel head coil. 7 T MRI exhibits benefits in comparison to imaging at lower magnetic field strengths, in particular improved signal to noise ratio and higher sensitivity (Bandettini et al., 2012; Sladky et al., 2013). Measurement time slots were between the hours of noon and 5 pm. The acquisition parameters applied for fMRI measurements were defined as previously described (Hahn et al., 2013). An echo-planar imaging sequence was utilized, where TE/TR = 23/1400 ms, which resulted in 32 axial slices with a voxel size of 1.5·1.5·2 mm plus 1 mm slice gap (matrix = 128·128). To ensure optimal coverage of the brain, the axial orientation of the field of view was parallel to the line between anterior and posterior commissures. In order to reduce ghosting artifacts, eyes were excluded from the field of view, hence, the slab was tilted more if required. Resting state fMRI was acquired over the course of 6 min. Subjects were instructed to focus their view on a white crosshair on a dark background, to remain in an awake yet relaxed state, and not to focus their thoughts on anything in particular ("let their thoughts wander").

Spatial normalization

Spatial normalization of fMRI data was carried out using SPM8 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm) using default parameters, unless otherwise specified. Slice timing correction, using the middle slice as the reference (Sladky et al., 2011), and motion correction, using the mean image as the reference, were performed. Furthermore, fMRI data was spatially normalized to Montreal Neurological Institute (MNI) stereotactic space using scanner-specific EPI templates (Sladky et al., 2013) and spatial smoothing with an 8 mm Gaussian kernel was performed.

Neuropsychological tests

Neuropsychological Tests were performed at MRI1, MRI2, and MRI3 in order to evaluate changes to empathy during cross-sex hormone treatment and gender transition. To investigate changes to emotional contagion we used the ECS, which assesses the extent to which persons are receptive to experiencing other's emotions and includes subscores of five basic emotions: "happiness," "love," "fear," "anger," and "sadness" (Doherty, 1997). The BVAQ is a test for alexithymia, which is associated with deficits in emotion recognition (Vorst and Bermond, 2001), a prerequisite for empathy (Bird and Viding, 2014). BVAQ scores were summed to derive the total alexithymia score, with high BVAQ scores indicating proneness for alexithymia (Vorst and Bermond, 2001).

Assessment of hormone levels

Blood draws were performed before each MRI visit in order to determine serum levels of testosterone and estradiol. Plasma levels were analyzed at the Clinical Institute for Medical and Chemical Laboratory Diagnostics at the Medical University of Vienna (KIMCL, http://www.kimcl.at).

Resting state fMRI analyses

Resting-state fMRI data was analyzed as described previously (Hahn et al., 2013) using Matlab R2011a. In summary, linear regression against motion parameters, white matter, and ventricles, followed by band-pass filtering (12-term finite impulse response filter, $0.007 < f < 0.08$ Hz), were used to remove possible confounding signals. Global signal correction was not performed because of issues regarding the interpretation of anti-correlations and between-group differences (Saad et al., 2012; Weissenbacher et al., 2009). A symmetrical version of the automated anatomical labeling atlas (AAL) (Tzourio-Mazoyer et al., 2002) was used to define 89 regions of interest (ROIs) (Savli et al., 2012). For each subject, functional connectivity matrices where computed between all ROI pairs by calculating the cross-correlation coefficient between the BOLD signal time courses from each pair of ROIs.

Network based statistics

Functional connectivity was assessed with NBS and computed using the NBS toolbox for Matlab (Zalesky et al., 2010). In contrast to seed based analyses methods, NBS does not require a priori selection of hubs or networks (Cole et al., 2010). In short, NBS analysis of functional connectivity data draws statistical inference on all networks throughout the entire brain. NBS is performed in two steps. First, each connection in the connectivity matrix is separately tested for significance (here: $p < 0.001$ uncorrected connection-level). Second, the resulting networks built by suprathreshold connections are again tested for significance (here: $p < 0.05$ FWE-corrected network-level). Although NBS is different to mass univariate analysis often carried out in fMRI, this process can be seen in analogy with cluster-level correction (Zalesky et al., 2010). Here, each voxel is first tested separately (voxel-level, usually $p < 0.001$ uncorrected), followed by testing of the spatial extent of the cluster, which comprises the suprathreshold voxels (cluster-level, usually $p < 0.05$ FWE-corrected).

Group differences between- and longitudinal changes to rsFC were assessed because hormone profiles differ between groups and change due to cross-sex hormone treatment. Therefore, time and group effects on rsFC may imply an influence of sex-steroid hormones. Group differences in rsFC at baseline were assessed using ANOVA (analyses of variance) and post hoc t-tests, which were corrected for multiple comparisons using NBS as described above ($p < 0.05$ FWE-corrected network level). In a next step, longitudinal changes to rsFC were assessed in the network showing the strongest difference between groups in the 15 MtF and 20 FtM in which longitudinal data was available. Functional connectivity strength was extracted as the sum of all connections for this network and repeated measures ANOVA was calculated in Matlab separately for each transgender group using time (MRI1, MRI2, MRI3) as factor.

Statistical analyses of behavioral and hormone data

ANOVA using group as factor (MtF, FtM, MC, FC) was performed to verify age-matching of groups, as well as to compare available (20 MtF, 11 MC, 22 FtM, 11 FC) plasma estrogen and testosterone levels at baseline to demonstrate that groups with the same biological sex did not differ. Linear mixed models analysis, with group (MtF, FtM) and time (MRI1, MRI2, MRI3) as fixed factors and subjects as the random factor was used to assess the effect of cross-sex hormone therapy on sex-steroid hormone plasma levels (estrogen, testosterone) as well as group differences and longitudinal changes to the empathy scores ECS and BVAQ in longitudinally assessed subjects. Each group was then assessed in a separate model and post hoc pairwise comparisons were performed using t-tests and corrected for multiple comparisons using the Bonferroni procedure. For all mixed models analyses covariance structure was defined based on Akaike's information criterion.

Correlation analysis was then performed in the group(s) that showed a change over time in order to detect whether observed changes were related to sex-steroid hormones or empathy. Change in sex-steroid plasma levels and change in empathy scores were correlated with change in rsFC within the network(s) elucidated with repeated

measures ANOVA. As mentioned above, functional connectivity strength was extracted from entire networks with significant group differences. To assess an influence of sex-steroid hormones, change in rsFC strength was correlated with change in estradiol and testosterone plasma levels between MRI1, MRI2, and MRI3. In addition, in order to detect an influence of empathy on rsFC, changes in BVAQ and ECS subscores were correlated with change in rsFC. Statistical Analyses employed SPSS version 19.0 for Windows (SPSS Inc., Chicago, Illinois; www.spss.com). For NBS data, as mentioned above, the significance threshold was set at $p < 0.001$ uncorrected at the connection-level followed by $p < 0.05$ FWE-corrected at the network-level. ANOVA and pairwise comparisons between groups were FWE-corrected at network level. When specific networks were assessed, i.e. in repeated measures ANOVA, t-tests between time-points, and correlation analysis, significance was limited to the connection level.

Results

Subjects

ANOVA showed no significant difference in age at MRI1 between the groups.

Hormones

At baseline neither MtF (mean estrogen ng/mL, mean testosterone ng/mL = 0.03, 5.67) and MC (0.03, 5.84) nor FtM (0.12, 0.38) and FC (0.09, 0.32) differed in plasma estrogen or testosterone levels.

Cross-sex hormone treatment resulted in a change in sex-steroid hormone plasma levels. For testosterone, linear mixed model analysis revealed no main effect of group or time, though a strong interaction between group and time was detected ($F_{2,41.9} = 57.61$, $p < 0.001$). In MtF, post hoc t-tests showed a significant reduction in testosterone plasma levels from MRI1 to MRI2 and MRI1 to MRI3 (both $p < 0.001$), though not from MRI2 to MRI3. In FtM, testosterone plasma levels increased significantly from MRI1 to MRI2 and from MRI1 to MRI3 (both $p < 0.001$), though not from MRI2 to MRI3. Similarly, mixed model analysis showed no main effect of group or time on estrogen plasma levels, though an interaction between group and time was detected ($F_{2,29.0} = 5.74$, $p < 0.01$). In MtF, post hoc t-tests showed an increase in estrogen plasma levels from MRI1 to MRI2 ($p < 0.01$) and from MRI1 to MRI3 ($p < 0.05$), though not from MRI2 to MRI3. In FtM no changes in estrogen level were observed. In summary, testosterone levels increased in FtM and decreased in MtF while estrogen levels increased in MtF but were unchanged in FtM (Fig. 1).

Empathy scores

Neuropsychological scores reflecting empathy showed group differences and changed over the course of treatment. Linear mixed models analysis revealed a main effect of time on BVAQ ($F_{2,31.8} = 13.68$, $p < 0.001$), with BVAQ decreasing from MRI1 to MRI2 ($p < 0.001$) and increasing from MRI2 to MRI3 ($p < 0.001$) when all transgender subjects were analyzed. Post hoc t-tests showed that BVAQ differed between MRI1 and MRI2 and between MRI2 and MRI3 (both $p < 0.001$) in MtF. For the ECS subscore "happiness" no effects of group, time or interaction effects were elucidated. Analysis of the subscore "love" showed an interaction effect ($F_{2,49.7} = 6.48$, $p < 0.01$). At MRI3 a group effect ($F_{1,30.0} = 5.30$, $p < 0.05$) was shown with higher scores in MtF than in FtM. For the subscale "fear" a main effect of group was elucidated ($F_{1,32.1} = 7.95$, $p < 0.01$) with higher scores in MtF than in FtM. This group effect was significant at MRI1 ($F_{1,32.0} = 11.84$, $p < 0.01$) and MRI2 ($F_{1,32.0} = 6.07$, $p < 0.05$) as well as trend-level significant at MRI3 ($F_{1,30.0} = 4.09$, $p = 0.052$). MtF showed higher scores than FtM at both time points. For the subscore "anger" a group effect ($F_{1,32.2} = 12.91$, $p = 0.001$) with higher scores in MtF than in FtM, was also significant at MRI1 ($F_{1,31.0} = 10.66$, $p < 0.01$), MRI2 ($F_{1,32.0} = 6.76$, $p < 0.05$), and MRI3 ($F_{1,30.0} = 12.55$, $p = 0.001$). For the subscore "sadness" a group effect ($F_{1,33.1} = 8.06$, $p < 0.01$) with higher scores in MtF than FtM was significant at MRI2 ($F_{1,33.0} = 5.8$, $p < 0.05$) and MRI3 ($F_{1,30.0} = 10.42$, $p < 0.01$) and trend-wise significant at MRI1 ($F_{1,33.0} = 3.45$, $p = 0.07$), with higher scores in the MtF group (Fig. 2).

Network based statistics

At baseline, ANOVA of rsFC assessed by NBS revealed a significant group effect within a network clustered around the supramarginal gyrus (SMG, $p < 0.05$ FWE-corrected, for regions and connections see Fig. 3A). In addition, post hoc t-tests between all groups revealed that MtF showed lower connectivity within the SMG network than FC and MC, with the largest difference seen between MtF and FtM (all $p < 0.05$ FWE-corrected, Fig. 3B). MC vs FC, FtM vs FC, and FtM vs MC did not differ in the SMG network ($p > 0.05$). The strongest difference in rsFC was found between the MtF and FtM groups (for regions and connections see Fig. 4A).

As the comparison of rsFC between MtF vs FtM showed the strongest difference, the network isolated in this contrast was used for subsequent longitudinal assessment of connectivity strength with repeated measures ANOVA (Fig. 4A) within the FtM and MtF groups. A main effect of time was found within the MtF group ($p < 0.05$). Post hoc t-tests demonstrated a significant increase in MtF from MRI1 to MRI2 and MRI1 to MRI3 ($p < 0.05$) and a trend-level increase from MRI2 to

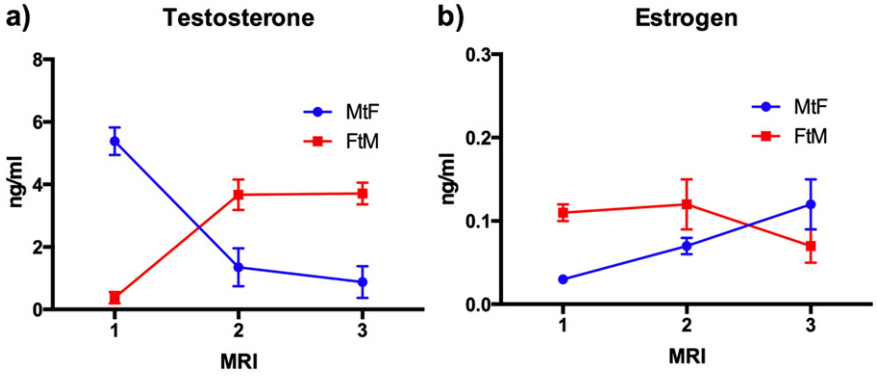

Fig. 1. Sex-steroid hormone plasma level changes over gender transition. A) In MtF, testosterone fell from MRI1 to MRI2 and MRI1 to MRI3 (both $p < 0.001$), though not from MRI2 to MRI3. In FtM, testosterone increased from MRI1 to MRI2 and from MRI1 to MRI3 (both $p < 0.001$), though not from MRI2 to MRI3. Values available in: MtF MRI1 n = 15, MRI2 n = 13, MRI3 n = 15; FtM MRI1–2 n = 18, MRI3 n = 15. B) In MtF, estrogen increased from MRI1 to MRI2 ($p < 0.01$) and from MRI1 to MRI3 ($p < 0.05$), though not from MRI2 to MRI3. FtM showed no significant changes to estrogen levels over time. Values available in: MtF MRI1 n = 15, MRI2 n = 13, MRI3 n = 15; FtM MRI1–2 n = 18, MRI3 n = 15. Dots/squares indicate mean. Whiskers show ±SEM.

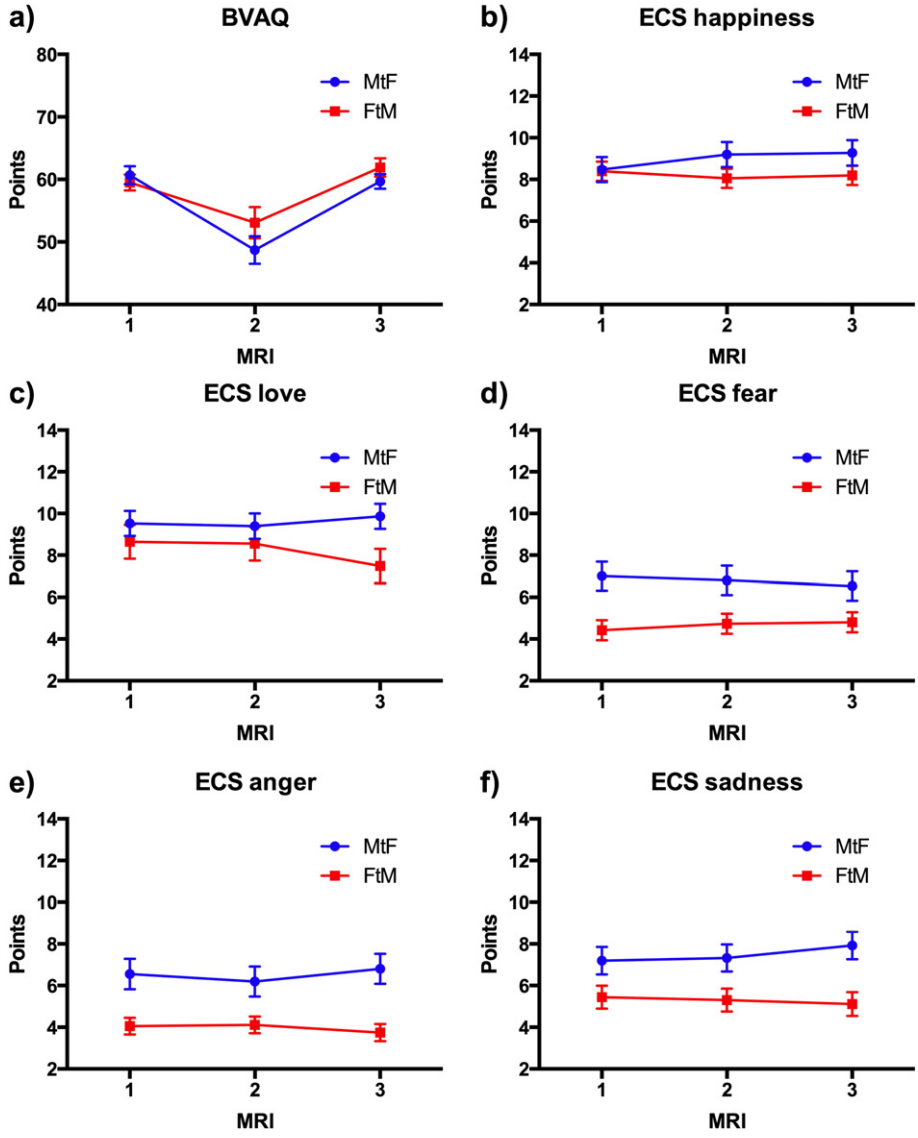

Fig. 2. Linear mixed models analyses of empathy scores. A) BVAQ. A main effect of time was shown ($F_{2,31.8} = 13.68$, $p < 0.001$), with scores decreasing from MRI1 to MRI2, and increasing from MRI2 to MRI3 (both $p < 0.001$). In MtF, scores decreased from MRI1 to MRI2 and increased between MRI2 and MRI3 (both $p < 0.001$). In FtM scores only increased from MRI2 to MRI3 ($p < 0.05$). Values available in: MtF MRI1 n = 14, MRI2–3 n = 15; FtM MRI1–2 n = 20, MRI3 n = 17. B) ECS happiness. No effects of group, time, nor interaction effects were elucidated. Values available in: MtF MRI1–3 n = 15; FtM MRI1–2 n = 20, MRI3 n = 17. C) ECS love. An interaction effect was shown ($F_{2,49.7} = 6.48$, $p < 0.01$). At MRI3 a group effect was shown ($F_{1,30.0} = 5.30$, $p < 0.05$), with higher scores in MtF than in FtM. In FtM, scores decreased from MRI1 to MRI3 ($p < 0.01$) and from MRI2 to MRI3 ($p < 0.05$). Values available in: MtF MRI1–3 n = 15; FtM MRI1–2 n = 20, MRI3 n = 17. D) ECS fear. A main effect of group was shown ($F_{1,\ 32.1} = 7.95$, $p < 0.01$), with higher scores in MtF than in FtM. This group effect was significant at MRI1 ($F_{1,32.0} = 11.84$, $p < 0.01$) and MRI2 ($F_{1,32.0} = 6.07$, $p < 0.05$), with MtF showing higher scores than FtM. Values available in: MtF MRI1–3 n = 15; FtM MRI1–2 n = 19, MRI3 n = 17. E) ECS anger. A group effect was shown ($F_{1,32.2} = 12.91$, $p = 0.001$), with higher scores in MtF than in FtM, which was also significant at MRI1 ($F_{1,\ 31.0} = 10.66$, $p < 0.01$), MRI2 ($F_{1,32.0} = 6.76$, $p < 0.05$), and MRI3 ($F_{1,30.0} = 12.55$, $p = 0.001$). Values available in: MtF MRI1 n = 14, MRI2–3 n = 15; FtM MRI1–2 n = 19, MRI3 n = 17. F) ECS sadness. A group effect was shown ($F_{1,33.1} = 8.06$, $p < 0.01$), with higher scores in MtF than FtM, which was significant at MRI2 ($F_{1,33.0} = 5.8$, $p < 0.05$) and MRI3 ($F_{1,30.0} = 10.42$, $p < 0.01$). Values available in: MtF MRI1–3 n = 15; FtM MRI1–2 n = 20, MRI3 n = 17. Dots/squares indicate mean. Whiskers show ±SEM.

MRI3 ($p < 0.01$) (Fig. 4B). SMG network connectivity did not change over time in FtM ($p > 0.05$).

The findings in the network around the SMG were confirmed when using independent samples for baseline and longitudinal effects (Fig. S1). Furthermore, the specificity of rsFC data was verified through utilization of control networks (Supplement).

In summary, MtF differed from all other groups within a network around the SMG. Gender transition, induced via cross-sex hormone treatment, resulted in assimilation of the connectivity strength of MtF to that of the other groups within the SMG network (Fig. 4B). FtM showed no significant change over time in this network.

Correlational analyses

Sex-steroid hormones

As described above, the MtF group was shown to be significantly different from all other groups at baseline, yet assimilated to the other groups over the course of treatment in connectivity strength within a network clustered around the SMG. While at baseline connectivity in this network was lower in MtF than in all other groups, it increased over the course of cross-sex hormone treatment. In order to assess if this effect was directly related to cross-sex hormone treatment, correlational analyses were performed between

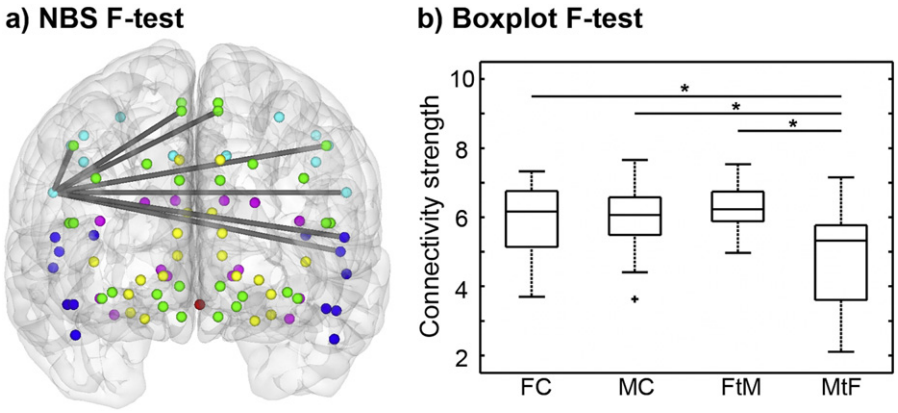

Fig. 3. MtF differ from all other groups in a rsFC network clustered around the SMG at baseline. A) ANOVA of rsFC assessed by NBS revealed a significant group effect within a network clustered around the left SMG (9 regions, 8 connections, $p < 0.05$ FWE-corrected). This includes connections to the right SMG, superior temporal cortex (right), rolandic operculum (right), postcentral- (left), precentral- (bilateral), and supplementary motor cortices (bilateral). Points indicate brain regions (green: frontal-, blue: temporal-, cyan: parietal-, magenta: occipital-, yellow: limbic/subcortical- regions), lines indicate connections. B) Pairwise analysis revealed that MtF showed significantly lower rsFC in this network than all other groups. *$p < 0.05$ FWE-corrected.

rsFC strength in the network we assessed longitudinally, and plasma hormone levels. The change in testosterone and estrogen hormone plasma levels did not correlate with the change in connectivity strength within the network ($p > 0.05$).

Empathy

To assess whether changes in rsFC observed within the SMG network in MtF across gender transition could be related to changes in empathy, changes in rsFC assessed via NBS were correlated with changes in empathy scores. Change in BVAQ from MRI1 to MRI2 correlated with change in SMG network connectivity strength between these two time-points ($r = 0.61$, $p < 0.05$). Furthermore, change within the ECS sub-score "fear" correlated with change in connectivity from MRI1 to MRI2 ($r = -0.68$, $p < 0.01$). Change to the other ECS scores did not correlate with rsFC changes.

Explorative correlational analyses revealed that neither change in ECS "fear" nor change in BVAQ correlated with change in estrogen or testosterone between the three fMRI measurements.

Discussion

This study investigated rsFC in a large group of transgender subjects undergoing cross-sex hormone treatment as part of gender transition to demonstrate that 1) transgender persons show unique rsFC patterns in a network related to empathic processing and 2) that the rsFC changes shown within this network over gender transition are likely related to changes in empathy rather than changes to sex-steroid hormones.

We isolated a rsFC network clustered around the SMG that differed significantly between MtF, FtM, MC, and FC at baseline before start of cross-sex hormone therapy and was shown to change in the MtF group over the course of cross-sex hormone treatment and gender transition. At baseline, MtF showed lower rsFC within the SMG network than all other groups. Interestingly, the low rsFC strength found in this network in MtF assimilated to that of the other groups after induction of cross-sex hormone treatment. One may theorize that the hormone changes displayed by MtF, specifically increasing estrogen or decreasing testosterone levels, may underlie this pattern. Yet, in our study a direct correlation between rsFC changes observed in the MtF group and change in plasma levels of sex-steroid hormones induced by cross-sex hormone treatment could not be elucidated.

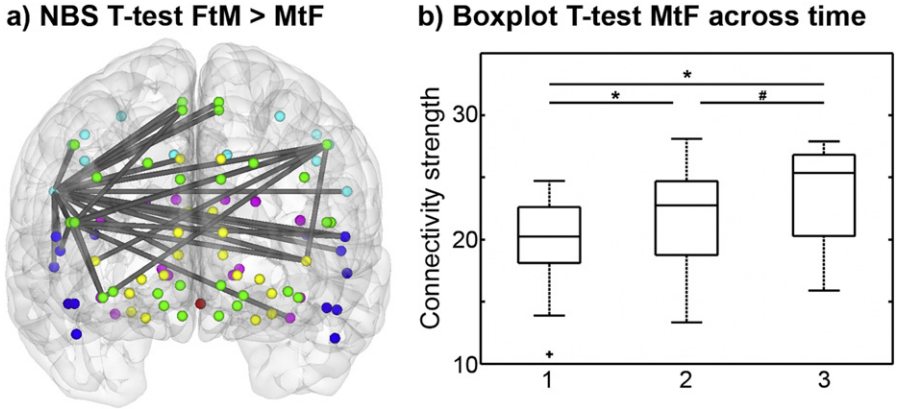

Fig. 4. MtF assimilate to all other groups in a rsFC network clustered around the SMG over the course of gender transition. A) The network elucidated in the comparison of MtF and FtM (25 regions, 33 connections) included connections between the SMG (bilateral), superior- (bilateral), and middle temporal cortices (left), rolandic operculum (bilateral), Heschl gyrus (bilateral), insula (bilateral), thalamus (left), cingulum (left), and fusiform gyrus (right). In addition, this network included the post- (bilateral) and pre-central cortices (bilateral), the paracentral lobule (bilateral), the supplementary motor area (bilateral), as well as the pars orbitalis (left), pars triangularis (left), and pars opercularis (left) of the inferior frontal cortex. Point color code same as in Fig. 3, lines indicate connections. B) The SMG network elucidated in the FtM vs MtF comparison was used for longitudinal analysis. Repeated measures ANOVA revealed that rsFC increased significantly in MtF over the course of cross-sex hormone treatment, hereby assimilating to baseline rsFC of all other groups. *$p < 0.05$, #$p < 0.1$.

An influence of sex-steroid hormones on functional connectivity has been demonstrated in numerous emotional (Volman et al., 2011; Klapwijk et al., 2013) and cognitive processes including attention (Thimm et al., 2014), language (Weis et al., 2008), and spatial cognition (Weis et al., 2011). In contrast, the available literature on the influence of sex-steroid hormones on rsFC is limited in general and offers partially contradictory results. While some fMRI studies have demonstrated an influence of sex-steroid hormones on rsFC (Petersen et al., 2014; Arelin et al., 2015; Engman et al., 2016), a recent investigation disputes an influence by arguing that sex differences in rsFC are based on fundamental differences between the two sexes, rather than related to hormonal changes (Hjelmervik et al., 2014). While sex differences in connectivity within the fronto-parietal resting state network were shown, no influence of menstrual phase and therefore of sex-steroid hormone profile was found (Hjelmervik et al., 2014). Furthermore, sex-specific changes to the default mode network have been shown in alexithymia using MR spectroscopy hereby demonstrating the relevance of sex in the modulation of emotion processing's neuronal correlates (Colic et al., 2016).

In their rsFC paper, Hjelmervik et al. discuss that the literature demonstrates an influence of sex-steroid hormones on functional connectivity, but that evidence is limited for the resting state. This is interpreted to suggest that sex-steroid hormones must be particularly relevant to regulation of cognitive control, while modulation of connectivity during the resting state is low (Hjelmervik et al., 2014). Alternatively, if one assumes that undulation of sex-steroid hormone levels over the menstrual cycle does not influence rsFC, yet sex does (Hjelmervik et al., 2014), one may postulate that short- and long-term changes to- or phases with- particular sex-steroid hormone levels may have differential effects on brain organization. Along this line, the lack of a decisive effect of menstrual cycle has also been demonstrated in neuronal activity patterns associated with sexual arousal assessed with fMRI (Abler et al., 2013). Accordingly, sex-steroid hormones play a pivotal role in brain sexual dimorphism from a prenatal stage to puberty (Herting et al., 2012; Bao and Swaab, 2011). On the one hand, the role of sex-steroid hormones in neural development demonstrates the effect of stable sex-steroid hormone levels. On the other hand, the influence of sex-steroid hormones may be restriced to, or vary between, different developmental stages (Nguyen et al., 2013). In our case, neither 4 weeks nor 4 months of cross-sex hormone treatment resulted in changes to rsFC in our SMG network. Therefore, one may postulate that either sex-steroid hormones did not effect rsFC within the network we elucidated, or any possible effects were overridden by more pronounced effects dependent on other factors.

In this context, if biological sex, or more stayed, long-term hormone levels, were the decisive factor in the rsFC network elucidated in our study, we may expect MtF and MC not to differ at baseline. However, these two groups did show differential rsFC in the SMG network, suggesting that it is likely that our findings reflect differences in brain organization between transgender and cis-sexual individuals, at least in the MtF group. Along this line, unique brain structure and function in transgender individuals in comparison to cis-sexual controls has previously been demonstrated in structural connectivity (Hahn et al., 2015), functional connectivity (Manzouri et al., 2015) and rsFC (Lin et al., 2014) studies. Our results therefore expand on previous results demonstrating distinctive rsFC patterns in transgender individuals.

Previous studies link the network elucidated in our study to interpersonal emotion processing. The network that correlated with changes in ECS and BVAQ was clustered around the SMG, which has been implicated in various interpersonal processes, in particular perspective taking (Lamm et al., 2007), overcoming egocentricity (Silani et al., 2013), processing of cooperative behavior (Leube et al., 2012), social aspects of movement (Buccino et al., 2007; Newman-Norlund et al., 2010), and language production (Kraemer et al., 2014). In addition, the SMG is considered a candidate site for mirror neurons (MN), which not only show activity during an action, but also during observation of such tasks (Salmi et al., 2013; Iacoboni and Dapretto, 2006). The MN system (MNS) is discussed as a neuronal correlate for empathic processes in that it is activated during observation and imitation of emotion (Carr et al., 2003). The SMG network elucidated in our study also reflects the regions' role in processes related to or necessitating empathic capabilities, as it displays connectivity to regions involved in emotional (Radua et al., 2010), somatosensory (Lee et al., 2016) sensorimotor, and language processing (Horwitz and Braun, 2004).

The rsFC changes we observed in MtF in the SMG network may be related to changes in empathic processing occurring over the course of gender transition. On the one hand, MtF scored higher in the ECS subscores “fear,” “anger,” and trend-wise higher for “sadness,” at baseline suggesting increased propensity for emotional contagion. However, BVAQ did not show a consistent trend over time in that it decreased from MRI1 to MRI2 and increased from MRI2 to MRI3, while no effect of time was elucidated from MRI1 to MRI2 for the ECS subscore “fear.” Differential time and group effects of the ECS subscores and BVAQ may, on the one hand, be explained by the observation that BVAQ and ECS can be considered unique components integrated as part of a common process, more specifically empathy (Bird and Viding, 2014). Nevertheless, between MRI1 and MRI2, change in BVAQ and ECS “fear” showed positive and negative correlations with change in rsFC, respectively. The fact that BVAQ showed positive-, and ECS “fear” negative-, correlations with rsFC, though BVAQ decreased from MRI1 to MRI2 and ECS “fear” showed no change, may be explained by the fact that we found an association between empathy scores and rsFC, yet the changes to empathy scores over gender transition are not homogenous within the MtF group.

Lower BVAQ, understood as an increased capability for detection and interpretation of emotion (Vorst and Bermond, 2001), and higher ECS scores, understood as increased propensity for emotional contagion (Doherty, 1997), may be interpreted as signalizing increased capability for empathy (Bird and Viding, 2014). Therefore, our results support a negative relationship between empathy and rsFC in the described network. To date, most studies investigating such an influence demonstrate a positive association between empathy and rsFC (Otti et al., 2010; Takeuchi et al., 2014). Again, this contradiction brings to light the possibility that the cerebral organization of transgender individuals may be different from those of cis-sexual persons, also in regard to how they are modulated by, or interact with, variables.

It is plausible that the changes to empathy associated with rsFC in our study could be related to changes in sex-steroid hormones, as numerous studies have demonstrated a role of sex-steroid hormones in modulation of empathic behavior (Bos et al., 2012; Knickmeyer et al., 2006; Derntl et al., 2013) and its neuronal correlates (Witte et al., 2010). Hereby, sex-steroid hormones would have indirectly modulated rsFC. However, we did not find a correlation between sex-steroid hormones and any of the empathy scores, suggesting that the relationship between empathy and rsFC is independent of the sex-steroid hormones assessed.

Like Hjelmervik et al. (Hjelmervik et al., 2014), we demonstrated that the changes to rsFC observed in MtF over the course of gender transition were not related to sex-steroid hormone levels. One must of course consider, that the absence of a correlation does not necessarily rule out an effect of hormones, as a decisive effect of time on rsFC was shown. For example, although cross-sex hormone treatment offers a highly unique opportunity to investigate the influence of high-dose treatment and substantial changes to sex-steroid hormone profiles, it is associated with variability. We can therefore not exclude the possibility that sex-steroid hormones influence, but do not correlate with, rsFC. In addition, considering that participants were individually treated based on desired effects and side-effects, our treatment protocol did allow for some flexibility in dosage and exact combination of medications. This variability may of course be aggravated by differences in absorption and metabolism. In this context, if one proposes that the longitudinal changes we show highlight an effect of hormonal

treatment, it is likely a qualitative rather than a quantitative one. In addition, the length of the resting state fMRI data acquisition time period may be discussed as a possible limitation. In general, the ideal length of a resting state session is a contested matter. Though some studies have suggested that longer scan times may improve intersession reliability (Birn et al., 2013), others demonstrate that correlation strength (Van Dijk et al., 2010) and signal homogeneity (Zuo et al., 2013) are sufficient at 5 min. However, longer acquisition times are also likely associated with practical drawbacks, such as increased risk of vigilance changes or even sleep and also motion artifacts. In this regard, 6 min of resting state fMRI data acquisition time may be considered a compromise between benefits of longer measurement time-periods and possible disadvantages for data quality.

In summary, this study shows that rsFC patterns, specifically a pattern likely related to empathic functioning, is distinct in MtF transgender participants when compared to cis-sexual controls. This finding is in line with previous studies demonstrating unique brain organization in transgender persons (Lin et al., 2014), though it is the first study to show unique rsFC patterns in a network related to empathy. The changes demonstrated in this network in MtF over the course of gender transition are likely related to changes to empathic functioning, rather than changes in sex-steroid hormones. Our results demonstrate that the cognitive and emotional factors that change over gender transition, in our case empathy, likely demonstrate distinct neurobiological correlates.

Conflict of interest

The authors declare no conflict of interest in the context of this study. The ClinicalTrials.gov identifier for this study is NCT01292785.

M. Spies has received travel grants from AOP Orphan Pharmaceuticals AG, Janssen-Cilag Pharma Gmbh, and Eli Lilly, workshop participation from Eli Lilly, and speaker honoraria from Janssen-Cilag Pharma Gmbh. G.S. Kranz has received travel grants from Roche Austria GmBH and Pfizer. C. Kraus has received travel grants from Roche Austria GmBH and AOP Orphan Pharmaceuticals AG. D. Winkler has received speaker honoraria from Angelini, Bristol-Myers Squibb, Novartis, Pfizer, and Servier. S. Kasper has received grant/research support from Eli Lilly, Lundbeck A/S, Bristol-Myers Squibb, Servier, Sepracor, GlaxoSmithKline, Organon, and Dr. Willmar Schwabe GmbH & Co. KG, has served as a consultant or on advisory boards for AstraZeneca, Bristol-Myers Squibb, German Research Foundation (DFG), GlaxoSmithKline, Eli Lily, Lundbeck A/S, Pfizer, Organon, Sepracor, Janssen-Cilag Pharma Gmbh, and Novartis, and has served on speakers' bureaus for AstraZeneca, Eli Lilly, Lundbeck A/S, Servier, Sepracor and Janssen-Cilag Pharma Gmbh. R. Lanzenberger received travel grants and conference speaker honoraria from AstraZeneca, Lundbeck A/S, Roche Austria GmbH, Dr. Willmar Schwabe GmbH & Co. KG, AOP Orphan Pharmaceuticals, and Janssen-Cilag Pharma Gmbh.

Acknowledgments

This work was supported by a grant awarded to R. Lanzenberger by the Austrian Science Fund (grant number: P 23021). E. Comasco is supported by the Swedish Society of Medicine (SLS-331991). We thank E. Akimova, P. Baldinger, A. Höflich, A. Kautzky, A. Komorowski, U. Moser, J. Losak, D. Heeger, J. Unterholzner, J. Sinaj, M. Godbersen, R. Gmeinder, O. Keskin, M. Klöbl, C. Tempfer, J. Atanelov, and M. Küblböck for their medical, technical, and administrative support. This scientific project was performed with the support of the Medical Imaging Cluster of the Medical University of Vienna. The authors are especially grateful to all transgender subjects for participating in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2016.05.060.

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