Circulating androgens correlate with resting-state MRI in transgender men

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Abstract

Despite mounting evidence regarding the underlying neurobiology in transgender persons, information regarding resting-state activity, particularly after hormonal treatment, is lacking. The present study examined differences between transgender persons on long-term cross-sex hormone therapy and comparisons on two measures of local functional connectivity, intensity of spontaneous resting-state activity (low frequency fluctuations, LFF) and local synchronization of specific brain areas (regional homogeneity, ReHo). Nineteen transgender women (TW, male-to-female), 19 transgender men (TM, female-to-male), 21 non-transgender men (NTM) and 20 non-transgender women (NTW) underwent a resting-state MRI scan. The results showed differences between transgender persons and non-transgender comparisons on both LFF and ReHo measures in the frontal cortex, medial temporal lobe, and cerebellum. More interestingly, circulating androgens correlated for TM in the cerebellum and regions of the frontal cortex, an effect that was associated with treatment duration in the cerebellum. By comparison, no associations were found for TW with estrogens. These data provide first evidence for a potential masculinization of local functional connectivity in hormonally-treated transgender men.

Keywords: functional connectivity; testosterone; transsexual; hormone treatment; masculinization; androgenization

1. Introduction

Transgender persons are characterised by persistent cross-sex identification and uneasiness with their natal sex (APA, 2013). Consequently, transgender persons often desire cross-sex hormonal treatment and surgical reassignment. Despite the increasing awareness and progress surrounding surgical gender reassignment, there is still a need for better understanding the potential impact of sex hormones on the underlying neurobiology. Functional and structural neuroimaging (fMRI/sMRI) studies in transgender persons are still scarce but slowly increasing. These limited studies have focused on whether transgender persons resemble their natal sex or their gender identity in structural neuroanatomy (for a review see Saraswat et al., 2015) or sexually-dimorphic cognitive-affective functions (for a review see Smith et al., 2015). Studies on the effects of cross-sex hormonal treatment in transgender persons have revealed changes in cortical, subcortical, and ventricular volumes and thickness (Hulshoff Pol et al., 2006; Luders et al., 2009; Zubiaurre-Elorza et al., 2014). Moreover, in transgender men (female-to-male) androgen treatment increased white matter diffusivity in cortical and cortico-spinal tracts (Rametti et al., 2012). However, the potential consequences of androgen or estrogen treatment on basic neural synchrony are currently unknown.

Resting-state activity is defined as the intrinsic fluctuations in neural activation when the brain is not actively engaged in any task. This resting-state activity may inform on essential networks underlying a variety of cognitive-affective functions (Biswal et al., 2010; Fox et al., 2005). Presently, resting-state MRI (rsfMRI) mainly focuses on functional connectivity examining the inter-regional temporal correlation between distant brain regions (e.g., between the amygdala and the prefrontal cortex) that may be involved in a specific cognitive function (Biswal et al., 2010; Fox et al., 2005; Greicius et al., 2007). However, resting-state activity may also characterize the intensity or synchronization of baseline neural signals within a localized brain region, i.e., local functional connectivity, which has been shown to influence whole brain dynamics (Deco et al., 2014). Recent technical advances in the analysis of rsfMRI data have begun to map such local functional connectivity providing

new measures of signal intensity. These measures include low frequency fluctuations (LFF) and regional homogeneity (ReHo), i.e., local synchronization of the neural signal.

Indeed, it has been argued that low frequency fluctuations in rsfMRI record physiologically meaningful signs of intrinsic brain function and mark *the intensity of spontaneous resting-state neural activity* (Zhou et al., 2010). LFF analysis in clinical populations has indicated regionally-specific changes in resting-state activity in the prefrontal and temporal cortices in depressed patients relative to healthy comparisons (Liu et al., 2014). These findings were interpreted to reveal a possible underlying brain-behavior mechanism that may be indicative of excessive self-referential processing or deficits in affective regulatory control commonly seen in depression.

Complementary to LFF, regional homogeneity (ReHo) characterizes *the local synchronisation of spontaneous fMRI BOLD signals* and has been described as an index of local functional connectivity (Jiang and Zuo, in press). This method assumes similarity of the temporal hemodynamic characteristics of neighbouring voxels within a functional cluster, i.e., of a dynamic synchronization of a given brain region (Zang et al., 2004). Akin to LFF, ReHo studies in depression (Liu et al., 2010) or autism spectrum disorders (Paakki et al., 2010) have documented regional alterations in local synchronization in patients highlighting disorder-specific perturbations.

The extent to which sex hormones influence or alter such local synchronization has not yet been investigated. Previous reports find that frontal brain oscillatory coupling is associated with testosterone levels in men (Miskovic and Schmidt, 2009). Similarly, studies in women report that resting-state EEG varies with estrogen level, menstrual cycle phase, and use of oral contraceptives (Brotzner et al., 2014), which influence inter-hemispheric transfer time of information (Hausmann et al., 2013). Interestingly, some changes in the oscillatory pattern of the sleep EEG have been documented in transgender persons on cross-sex hormone therapy (Kunzel et al., 2011) indicating alterations in basic neural synchronization.

The goal of the present study was to compare the *intensity* (LFF) and *synchronization* (ReHo) of local functional connectivity using rsfMRI in transgender persons on cross-sex

hormone treatment and non-transgender comparisons. Moreover, the potential impact of hormone therapy was assessed by examining the influence of circulating sex hormones on resting-state activity. Based on limited available evidence (Kunzel et al., 2011; Rametti et al., 2012), we anticipated a shift toward resting-state activity consistent with their gender identity in transgender persons with treatment.

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2. Materials and Methods

2.1 Participants

Nineteen transgender women (TW, male-to-female, mean age =40.53 years, SD = 8.55), 19 transgender men (TM, female-to-male, mean age = 36.84 years, SD = 8.59), 21 non-transgender men (NTM, mean age = 32.57 years, SD = 9.88), and 20 non-transgender women (NTW, mean age = 34.6 years, SD =11.20) participated (Table1). All transgender persons (except one) were two years post gender reassignment surgery but all were receiving hormonal treatment; TM for a mean duration of 84.52 months (SD = 49.49 months) and TW for 81.56 months (SD=66.17 months). TM were receiving an injection with 1000mg of intramuscular testosterone undecanoate (Nebido®, Bayer, Germany, once every 10- 12 weeks, N=10), intramuscular testosterone esters (testosterone decanoate 100mg, testosterone isocaproate 60mg, testosterone fenylpropionate 60mg, testosterone propionate 30mg/ml, Sustanon 250®, MSD, The Netherlands, between ¾-2 ampoules every two weeks, N=8) or transdermal testosterone gel (Itnogen® 2% gel, 50 mg/day, N=1). TW were receiving 4mg (2x 2mg) oral estradiol valerate daily (Progynova ®, Bayer, N=7), transdermal 17-B estradiol patch 0.100 mg/24h (Dermestril®, Besins, Belgium, N=3), transdermal 17-B estradiol gel (2 x 2mg daily, N=7, Oestrogel®, Besins, Belgium or Estreva®, Besins, Belgium)

or contraceptive pill daily (N=1). Transgender persons were recruited through flyers and through the Department of Endocrinology of the Ghent University Hospital. Non-transgender comparison participants were recruited through word of mouth and through flyers that were distributed throughout the city of Ghent. The study was approved by the Medical Ethical Committee of Ghent University Hospital. All participants were screened for psychiatric pathology by a clinician (KW) using the MINI neuropsychiatric interview (Sheehan et al., 1997).

2.2 Resting-state MRI

Resting-state images (acquisition time 6:04 minutes) were acquired on a 3T Siemens Trio (TrioTim syngo MR B17, Siemens, Erlangen, Germany). The scanning parameters were as follows: TR/TE = 2000/27ms, FOV = 192mm, 34 slices, slice thickness = 3 mm. The first 6 scans were discarded to account for signal saturation effects. Participants were instructed to remain awake and relaxed with their eyes open and to fixate on a white cross on a projection screen. For normalization purposes, a high resolution anatomical MPRAGE (acquisition time 5:14 minutes) was acquired with flip angle = 9°; field of view (FOV) = 256 mm; repetition time (TR) = 2250 ms; echo time (TE) = 4.18ms.

2.3 Image processing

Data were pre-processed using the Data Processing Assistant for Resting-State fMRI (DPARSF, V2.0_101025, http://www.restfmri.net), based on SPM 8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) and the Resting-State fMRI Data Analysis Toolkit (REST, V1.5_101101, http://www.restfmri.net). Further pre-processing included slice timing correction, head motion correction, spatial normalisation, and smoothing. The standard EPI template from the Montreal Neurological Institute (MNI) was used for spatial normalisation. Data were spatially smoothed using a 4mm full-width half maximum (FWHM) Gaussian Filter. Finally, linear detrending and temporal band pass (0.01-0.08 Hz) filtering

were performed to remove low frequency drifts and physiological high-frequency noise (Cordes et al., 2001).

2.4 LFF analysis

LFF was calculated using the REST toolkit. The LFF at each voxel was computed for each subject, and it was further divided by the global mean value to reduce the global effects of variability across participants (Zang et al., 2007). Each LFF map was detrended and band pass filtered at the default frequency (0.01-0.08 Hz), and spatially smoothed with a Gaussian filter of 4 mm FWHM before analyses were run. Individual mean LFF images were then analysed in SPM8 with Group (TM, TW, NTM, NTW) as the main factor of interest and age as a covariate of no interest. Because this study aimed to examine the whole brain, we used a combined voxel and cluster-size thresholding approach to correct for multiple comparisons using Monte Carlo Simulations (with the programme *AlphaSim* as implemented in AFNI, http://afni.nimh.nih.gov/afni/) with 10.000 iterations and a voxel-wise p-value set at .001. These simulations indicated that in order to control for a Type I error, a minimum cluster size of 47 voxels would be needed to accomplish a corrected alpha level of p=.05 (that is 47 contiguous voxels would occur less than 5% of the time by random noise alone assuming a group of highly significantly activated voxels set at an individual voxel-wise threshold of p=.001).

2.5 ReHo analysis

The ReHo maps were generated using the REST toolkit. Kendall's coefficient of concordance (KCC) (Kendall and Gibbons, 1990) was used to measure the similarity of the time series within a functional cluster based on the regional homogeneity hypothesis (Zang et al., 2004). The individual ReHo maps were generated in a voxel-wise fashion, with 27 nearest neighbouring voxels defined as a cluster. A predefined mask (made with the MNI template to assure matching with the normalization step), in the REST software was used to remove non-brain tissue. The ReHo maps were divided by their own KCC value within the mask for

standardisation purposes (Wu et al., 2009). The ReHo maps were spatially smoothed with a Gaussian filter of 4mm FWHM. Age was used as a covariate of no interest in the analyses. Individual mean ReHo images were then analysed in SPM8 with Group (TM, TW, NTM, NTW) as the main factor of interest and age as a covariate of no interest. As above, Monte Carlo Simulations (*AlphaSim,* 10.000 iterations, voxel-wise p=.001*)* were run to determine the minimum cluster size needed to correct for Type I errors. Simulations for ReHo analysis indicated that a minimum cluster size of 57 contiguous voxels was needed to accomplish a corrected alpha of p=.05.

2.6 Analyses of correlations between sex hormones and LFF and ReHo

To examine how circulating sex hormones correlated with LFF and ReHo measures, SPM ANOVA matrices were set up that examined the correlations between TM and NTM for luteinizing hormone, testosterone, and androstenedione, and between TW and NTW for luteinizing hormone, E1, and E2 for LFF and ReHo, respectively. Additional contrasts were not done based on the logic that female sex hormones were suppressed in TM and male sex hormones were suppressed in TW. Such comparisons between transgender persons with their natal sex would have unlikely yielded any significant effects. The respective combined cluster- and voxel-wise thresholds as in the main analyses were used to correct for multiple comparisons. Moreover, as in the main analysis, age was covaried for in all correlations.

2.7 Hormonal assays

Venous blood was obtained and serum was stored at −80˚C until hormones were analyzed in one batch. Luteinizing hormone (LH) was measured by electrochemiluminiscence immunoassay (ECLIA) (Modular, Roche Diagnostics, Mannheim, Germany). The inter-assay CV was 2.19%. Estradiol (E2), estrone (E1), androstenedione, cortisol, and testosterone were determined using liquid chromatography tandem mass spectrometry (AB Sciex 5500 triple-quadrupole mass spectrometer; AB Sciex, Toronto Canada). The serum limit of quantification was 0.3 pg/mL for E2 and 0.5 pg/ml for E1, and the inter-assay CVs were 4% at 21 pg/mL for

E2 and 7.6% at 25 pg/mL for E1 (Fiers et al., 2012). Serum limit of quantification was 1 ng/dL (35pmol/L) for T, and the interassay CV was 6.5% at 3 ng/dL.

3. Results

3.1 Low frequency fluctuations (ALFF)

Transgender persons differed from their natal sex in low frequency fluctuations in the frontal lobe and the cerebellum. Specifically, relative to NTW, TM exhibited greater LFF in the left precentral gyrus (Fig 1, right panel) but smaller LFF in two areas of the cerebellum, the inferior semi lunar lobule and the pyramis. Conversely, TW showed greater LFF than NTM in parahippocampal gyrus but smaller LFF in the insula and postcentral gyrus. Looking at sex differences in non-transgender participants, similar to the finding in TM, natal women had larger LFF than natal men in the inferior semi-lunar lobule of the cerebellum (Table 2). There were no differences between TM and TW.

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3.2 Regional homogeneity (ReHo)

Mirroring the pattern observed in LFF, TM had smaller ReHo in the cerebellum (uvula) than NTW (Fig 2, top and bottom left). TM also differed from their gender identity by showing smaller ReHo than NTM in the auditory cortex, i.e., the transverse temporal gyrus. Similarly, TW differed from their gender identity by showing larger ReHo than NTW in the medial frontal gyrus. Consistent with the above findings, examination of sex differences revealed larger ReHo for natal women relative to natal men in the cerebellum (Fig 2, top and bottom left) but smaller ReHo for natal women in the frontal lobe, namely the pre- and postcentral gyri and the inferior frontal gyrus (Table 2, Fig 1 left and right panels). In the direct

comparison of both transgender groups, TM had larger ReHo than TW in the middle occipital gyrus.

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3.3 Analysis of circulating hormones with LFF and ReHo

3.3.1 LFF

The analysis of circulating hormone levels (testosterone, androstenedione, luteinizing hormone, E1 and E2) revealed some associations with LFF in a variety of regions. Both TM and NTM showed associations with androgens. In TM, testosterone and androstenedione were positively associated with greater LFF in the precentral gyrus (Fig 1 right panel). Moreover, androstenedione levels were negatively associated with LFF in the cerebellar uvula and pyramis. In NTM, lower testosterone was associated with larger LFF in superior frontal gyrus and cingulate gyrus while higher androstenedione levels were associated with larger LFF in lingual gyrus, middle occipital gyrus and uncus. In contrast to androgens, there were few associations for E1, E2, or LH in NTW or TW. Only E2 was significantly positively correlated with LFF in the inferior parietal lobule in NTW (Table 3).

3.3.2 ReHo

Regarding ReHo a strong effect of circulating androgens on the cerebellum became apparent in TM and NTM. In TM both testosterone and androstenedione were negatively correlated with regional homogeneity in the uvula and pyramis. In NTM this negative association was apparent for androstenedione in the cerebellar tonsil (Table 3, Fig 2, bottom right). In addition, NTM also showed a positive association with ReHo in the middle temporal gyrus. No associations emerged for TW or NTW.

3.3.3 Examination of treatment duration on the observed effects

To examine whether treatment duration was associated with the observed effects in LFF and ReHo, we conducted an additional exploratory analysis using the cluster of significant correlation of androstenedione in the cerebellum in TM as a mask and checked for an effect of treatment duration (in months) as a covariate in this region with a more lenient threshold of p =.005. This analysis revealed a significant positive association with treatment in TM in the nodule of the cerebellum [k = 27, t = 4.00, xyz = 0 -58 -25]. There was no association with treatment in the precentral gyrus cluster.

3.4 Exploratory analysis of psychiatric psychopathology

To assess the extent to which the findings could have been driven by psychiatric comorbidity, we re-ran analyses but with the 5 participants (2 TM, 3 TW) with the most psychopathologies (2 or 3 comorbidities each) excluded. Effects remained stable albeit with somewhat smaller cluster sizes due to the reduced statistical power.

4. Discussion

This study investigated differences in local functional connectivity as measured by intensity (LFF) and synchronization of spontaneous fluctuations (ReHo) of brain activity in transgender persons. Based on limited available evidence (Kunzel et al., 2011; Rametti et al., 2012), we had hypothesized a shift toward gender identity in resting-state activity in transgender persons on cross-sex hormone therapy. Three main findings pertinent to this study hypothesis emerged. First, consistent with predictions, TM differed from their natal sex (i.e., non-transgender women) in the cerebellum (ReHo/LFF) and the frontal cortex (LFF), which was supported by correlations with circulating androgens in these regions. Second, the observed patterns in TM were consistent with sex differences between natal men and natal women in resting-state activity in the cerebellum and the frontal lobe. However, third and contrary to the hypothesis, TM and TW also differed from their gender identity in ReHo in the medial frontal lobe and primary auditory cortex.

The main finding emerging from this study was an association of androgens with resting-state activity in the cerebellum in TM paralleled by sex differences in these regions between non-transgender men and non-transgender women. LFF and ReHo were both larger in this region in non-transgender women relative to TM. Moreover, the negative correlation of androgens in TM in large areas of the cerebellum approximated resting-state activity of natal men. The cerebellum is mostly known for its role in motor control and motor learning although its putative role in cognitive processes is also slowly being acknowledged (Van Overwalle et al., 2014). Prior reports have indicated a negative association of sex chromosome number but not sex hormone levels with cerebellar volume (Lentini et al., 2012). The precise role of sex hormones in cerebellar development is currently under debate, recognizing on the one hand the role of the cerebellum as a target for estrogen (and progesterone) action (Hedges et al., 2012), but also suggesting little change with sex hormones in adulthood (Dean and McCarthy, 2008). In support of the latter hypothesis (Dean and McCarthy, 2008), we found no correlations of estrogens or LH in NTW or TW in the cerebellum. By contrast, androgens were negatively associated with resting-state activity in TM, with some correlations also being visible in NTM. Of note, although evidence linking androgens to the cerebellum is currently very limited, some correlational evidence links testosterone with increase in fine motor skills in high school students (Wegner et al., 2014), while in two cases cerebellar ataxia has been found in males with hypogonadism (Erdemoglu et al., 2000). Sadly, our data preclude a conclusion that differences in resting-state fluctuations in the brain between NTW and TM were due to the hormone treatment, given the lack of pre-scan data. However, the negative correlations in TM, i.e., smaller androstenedione levels in TM would resemble the resting-state patterns of natal women, would suggest sensitivity to activational effects of androgens in the cerebellum in adulthood. This is further supported by a sexually-dimorphic pattern between natal men and natal women in LFF and ReHo in the cerebellum in the present data.

Regional specificity in other brain areas also indicated differences between transgender persons and their natal sex. For example, TW differed from NTM in LFF in

structures of the medial temporal lobe, namely the parahippocampus. A body of work is slowly emerging that demonstrates associations between the parahippocampus and androgens in a variety of contexts. Studies in both healthy adults (Lentini et al., 2012) and boys with androgen excess (Mueller et al., 2011) have found a positive association between androgens and parahippocampal gyrus volume. Moreover, parahippocampal activity increased after sublingual testosterone administration in natal women during a spatial orientation task (Pintzka et al., 2016). Differences in LFF at-rest in this region might indicate, as in the cerebellum, regional sensitivity to activational effects of androgens that may further influence underlying cognitive-affective function.

A final area that deserves attention is the frontal cortex. Sexual dimorphism in the frontal cortex has been observed in a variety of settings. For example, inferior frontal gyrus volume (IFG, BA44/45) is modulated by the androgen receptor (AR) gene in women (Raznahan et al., 2010), IFG is larger in women than in men (Ruigrok et al., 2014), and is an area that displays sex differences in activation patterns in prefrontally-mediated tasks such as verbal fluency (Gauthier et al., 2009). In the present study, natal men relative to natal women and transgender men differed in resting-state measures in IFG thus being consistent with this prior work. Moreover, resting-state activity between men and women also differed in the left precentral gyrus (BA6), an area that has been found larger in men than in women (Ruigrok et al., 2014). Interestingly, whereas resting activity of the IFG differed between transgender men and their gender identity, in the precentral gyrus, transgender men differed from natal women in LFF, an effect that was supported by positive correlations with androgens in this structure. Taken together, the precise meaning of group differences in resting-state MRI remains to be determined. However, the present findings are consistent with theories that propose that differences in temporal dynamics of intrinsic brain activity may organise behaviour (Biswal et al., 2010; Fox et al., 2005) and the notion of sexual dimorphism in functional connectivity between distant brain regions (Biswal et al., 2010). Importantly, in the present context, they show that such intrinsic activity is sensitive to variation in gonadal hormones.

The present data extend a slowly mounting research agenda in transgender persons (e.g., Smith et al., 2015) and the search for a biological basis of gender identity (Saraswat et al., 2015). Importantly, the present study extends urgently needed work to examine the long-term effects of cross-sex hormone therapy in transgender persons. While cross-sectional data indicate potential long-term health changes (osteoporosis, thromboembolic, or cardiovascular events) during hormonal treatment (Wierckx et al., 2012), evidence of hormonal therapy on neuroanatomy is scarce. The little available evidence to date indicates alterations in ventricular volume and cortical thickness in various areas of the cortex (Zubiaurre-Elorza et al., 2014), increased diffusion in white matter structural connectivity (Rametti et al., 2012) and reduction of the availability of neurotransmitters (Fuss et al., 2015) with cross-sex hormone therapy. Whereas this prior literature indicated differences on structural anatomy with cross-sex hormone therapy, the present data revealed differences during spontaneous synchronized neural activity at-rest. Future work may need to examine to what extent these differences can characterize performance of task based neural activity.

Some important limitations require discussion. First, we acknowledge the small sample size of participants in the study. However, using a combination of a voxel-wise and cluster-wise correction to control for multiple comparisons at the whole-brain level, we were nonetheless able to detect a variety of significant group differences indicating sufficient sensitivity of our data for the variables of interest. Yet, although participants were matched for education level and ethnicity (all were of Caucasian ethnicity), the representativeness of the findings have to be taken with caution given the relatively small number of comparison participants and the potential bias of participants willing to undergo an MRI study. Second, the present study examined resting-state transgender persons on hormonal treatment already living in their gender identity. Given that we were not able to acquire pre-hormonal treatment scans, we cannot make any strong inferences with regards to the causal nature of the hormonal treatment vs. inherent group differences. However, we hope that inclusion of correlations with circulating hormone levels mitigates this fact and provides valuable information on the possible effect of cross-sex hormone treatment on spontaneous brain

activity. Additionally, although the present study utilized state-of-the-art hormonal analyses providing great sensitivity with regards to hormonal assays, hormonal levels in the blood may not necessarily reflect hormonal levels in the brain. Given these limitations, the data are best seen in a larger context of converging studies examining the impact of sex hormones on brain structure and function beyond those of transgender persons such as endocrine conditions (Lentini et al., 2012; Merke et al., 2003; Mueller et al., 2009; Mueller et al., 2011; Skakkebaek et al., 2014) or general sex differences (Raznahan et al., 2010; Ruigrok et al., 2014). Finally, while much prior MRI work has invested effort to exclude transgender persons with psychiatric comorbidity, we decided to include all participants based on the notion that such a sample is more representative given a 70% prevalence rate of a current or lifetime psychiatric diagnosis in a large pan-European sample of individuals with gender dysphoria (Heylens et al., 2014). While some contribution of psychiatric comorbidity to the present findings cannot be completely ruled out, presence of psychopathology was relatively low for a given diagnosis. This was based on the fact that the greatest contribution of psychiatric comorbidity was driven by 5 individuals (2 TM and 3 TW) who had at least 2 or 3 comorbid affective disorders each (depression, dysthymia, generalized anxiety disorder, social phobia, panic disorder, agoraphobia). Moreover, after excluding these 5 individuals, effects remained stable. This would indicate that psychopathology did not, or at least very little, influence our findings.

5. Conclusions

In summary, to our knowledge, this is the first study to characterize resting-state activity and local functional connectivity in transgender persons. The main finding of this study was differences between TM and NTW in resting-state activity and associations of this activity with circulating androgens in the cerebellum and frontal cortex. The present data indicate that intrinsic brain activity is related to current sex steroid fluctuations. Future work will need to examine whether these perturbations in resting-state activity manifest in behavioural changes subserved by the affected neurocircuitry.

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Captions

Table 1. Demographic information as well age psychiatric comorbidity and hormonal levels for non-transgender men (NTM), non-transgender women (NTW), transgender men (TM) and transgender women (TW).

Table 2. Displays the significant group differences in low frequency fluctuations (ALFF) and regional homogeneity (ReHo). Only regions are listed that survived correction for multiple comparison, $p<.05$, corrected. Several structures are reported if peaks within a cluster were more than 8mm apart. Coordinates are MNI coordinates. ISLL – Inferior Semi-Lunar Lobule

Table 3. Displays the results from the correlational analysis, for each group separately, for ALFF and ReHo for testosterone (T), androstenedione (A), estrogen 1 (E1), estrogen 2(E2), and luteinizing hormone (LH). "+" indicates positive correlation and "-" indicates negative correlation for non-transgender men (NTM), non-transgender women (NTW), transgender men (TM) and transgender women (TW). Only regions that survived correction for multiple comparisons are listed ($p<.05$, corrected). Coordinates are MNI coordinates. Several structures are reported if peaks within a cluster were more than 8mm apart.

Figure 1. Left panel. Figure illustrates larger LFF in NTM relative to TM (in green) and larger ReHo in NTM relative to NTW (in red) in the right inferior frontal gyrus (IFG). The yellow color illustrates the overlap between the two conditions suggesting that TM are more similar to their natal sex than their gender identity in this region. **Right panel**. This figure shows the larger ReHo activity in NTM relative to NTW (in green), the larger LFF in TM relative to NTW (in red) and the significant positive correlation of LFF activity with testosterone levels in TM (in blue) in the left precentral gyrus. This figure illustrates that TM resemble their gender identity more than their natal sex in this region, an effect that correlated with testosterone levels in this group. The upper black circle shows the overlap (in pink)

indicating a significant correlation in the same region where TM differ from NTW. The lower circle illustrates that the difference between NTM and NTW (in green), between TM and NTW (in red) and the correlation of LFF with testosterone in TM (in blue) are all located within the same vicinity of the precentral gyrus with some overlap (pink and yellow colors). A = anterior, P = posterior, L = left, R = right, S = superior, I = inferior. Image was created with MRIcron (http://people.cas.sc.edu/rorden/mricron/index.html) using SPM maps with a voxelwise threshold set at p=.001.

Figure 2. Top. Grey circles on axial slices illustrate the overlap (in grey) in the left and right cerebellum among the negative correlation of ReHo with androstenedione levels in TM (in green), the greater ReHo activity in NTW relative to TM (in blue), and the greater ReHo in NTW relative to NTM (in red). This figure illustrates that TM are more similar to their gender identity than their natal sex in ReHo activity, an effect that correlates with androstenedione levels in this cohort. Pink color denotes the overlap between red and blue and turquoise the overlap between green and blue. **Bottom left**. 3D rendered image of the same effect illustrated on the top. **Bottom right**. Correlation between the androstenedione levels in TM and extracted parameter estimates for ReHo activity (10mm sphere around MNI xyz [-12 -76 -23], cf. Table 3). Correlation stays significant if point at the top left is removed. Brain images were created with MRIcron and SPM maps with a voxelwise threshold of p=.001. A = anterior, P= posterior, L=left, R=right, S = superior, I = inferior. Numbers at the top indicate z coordinates.

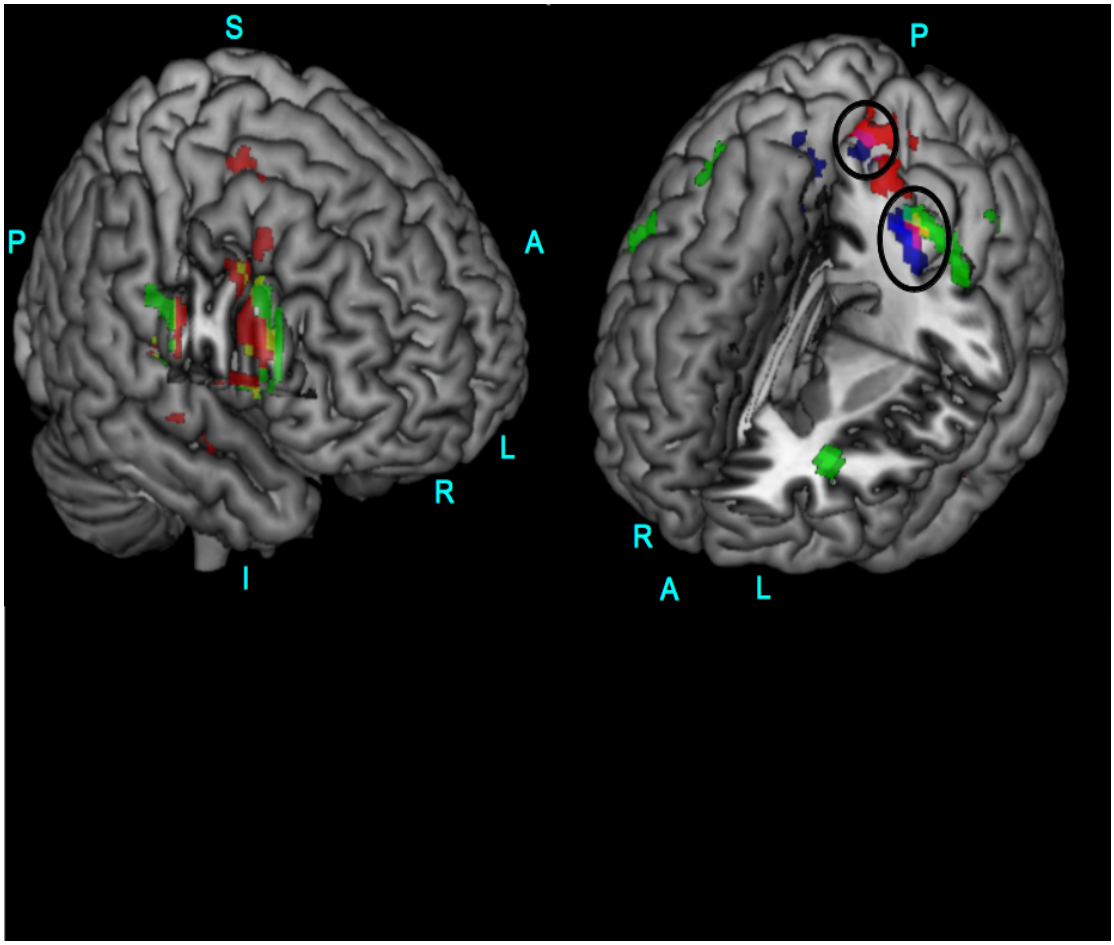
	NTM (N= 21)	NTW (N=20)	TM (N=19)	TW (N=19)	Signif.
Age in years (Mean/SD)	32.57 (9.88)	34.50 (11.20)	36.84 (8.59)	40.53 (8.55)	TW > NTM
Education (number of years/SD)	16.15 (2.21)	16.25 (2.55)	14.11 (2.99)	14.29 (2.87)	ns
Treatment duration (months/SD)			84.52 (49.49)	81.56 (66.17)	ns
Psychiatric comorbidity (MINI): N					
Depressive episode			2	2	
Dysthymia	-	-	4	2	
Hypomania	-	-	1	-	
Panic disorder	-	-	2	-	
Agoraphobia	-	1	1	3	
Social phobia	-	-	1	3	
OCD[a]	-	-	1	-	
PTSD[b]	-	-	-	2	
Alcohol/drug abuse	-	-	1	-	
Generalized anxiety	-	-	-	3	
Pain disorder	-	-	1	-	
Hormonal levels					
Testosterone (ng/dL)	456.37 (184.46)	27.24 (10.03)	841.37 (627.25)	14.47 (5.25)	
Androstenedione (ng/dL)	60.62 (20.10)	80.79 (36.29)	104.83 (47.92)	53.73 (19.13)	
E1 (pg/mL)	31.80 (21.18)	78.45 (63.12)	59.54 (21.96)	242.51 (423.76)	
E2 (pg/mL)	20.26 (8.07)	88.46 (96.18)	36.67 (16.55)	99.76 (122.65)	
Luteinizing hormone (LH) (mU/mL)	5.57 (2.26)	12.61 (13.70)	5.65 (8.59)	28.50 (15.96)	
Cortisol (µg/dL)	9.36 (3.91)	7.79 (3.34)	7.68 (3.27)	8.79 (3.63)	

[a]Obsessive compulsive disorder
[b]Post-traumatic stress disorder

Effect	Side	Region	Brodmann Area	Cluster size (K)	T value	Coordinates
LFF						
TW > NTM	L	Parahippocampal Gyrus	35	54	4.82	-15 -16 -29
TM > NTW	L	Precentral Gyrus	6	75	4.14	-24 -10 70
NTW > TM	L	Cerebellum – ISLL	-	614	5.89	-45 -79 -35
		Cerebellum - Tuber			5.56	-33 -76 -29
	R	Cerebellum - Pyramis	-	61	3.89	33 -76 -32
NTW > NTM	L	Cerebellum – ISLL	-	907	5.73	-45 -79 -35
	L	Lingual Gyrus	17		5.60	-18 -97 -17
	R	Cerebellum – ISLL	-		5.25	3 -67 -41
NTM > TW	L	Insula	13	47	4.22	-48 -19 22
	R	Postcentral Gyrus	3	78	3.95	63 -10 25
NTM > TM	R	Inferior Frontal Gyrus	44	65	4.57	60 17 13
ReHo						
TW > NTW	L	Medial Frontal Gyrus	9	97	4.25	-15 38 22
TM > TW	L	Middle Occipital Gyrus	37	113	4.01	-36 -64 1
NTW > NTM	L	Cerebellum – Tuber	-	1406	5.29	-24 -94 -32
	L/R	Cerebellum – Tonsil			4.98	0 -52 -44
	R	Cerebellum - ISLL			4.96	3 -67 -44
NTW > TM	L	Cerebellum – Uvula	-	1292	6.29	-24 -94 -29
	L	Cerebellum – Pyramis			5.92	-30 -82 -32
NTM > NTW	L	Precentral Gyrus	6	120	5.04	-36 -7 61
	R	Inferior Frontal Gyrus	45	73	4.47	57 20 19
	R	Postcentral Gyrus	3	63	4.27	63 -10 22
NTM > TM	R	Transverse Temporal Gyrus	42	74	4.60	66 -16 13

Hormone and directionality	Group	Hemis-phere	Region	BA	Cluster Size (k)	T Value	Coordinates
ALFF							
T +	TM	L	Precentral gyrus	6	101	9.39	-27 -16 55
T -	NTM	R	Superior frontal gyrus	10	82	5.37	18 62 7
		R	Cingulate gyrus	31	74	5.11	21 -43 40
A +	NTM	R	Lingual gyrus	19	137	7.08	21 -67 1
		R	Uncus	28	78	6.93	18 2 -32
		L	Middle occipital gyrus	18	211	6.81	-30 -82 -2
	TM	L	Precentral gyrus	6	91	7.49	-27 -16 55
		R	Rectal gyrus	11	64	6.28	9 29 -26
A -	TM	R	Cerebellum - Uvula		42[a]	4.16	36 -73 -26
		L	Cerebellum - Pyramis		39[a]	3.96	-12 -82 -29
E1			None				
E2 +	NTW	L	Inferior parietal lobule	40	65	4.91	-60 -37 37
LH	TM	L	Postcentral gyrus	3	47	4.98	-33 -34 49
REHO							
T -	TM	L	Cerebellum – Uvula		645	6.62	-9 -61 -26
		L	Cerebellum - Tuber			5.72	-54 -67 -26
A +	NTM	R	Middle temporal gyrus		61	4.10	39 -68 22
A -	NTM	L	Cerbellum - Tonsil		61	4.07	-21 -34 -35
	TM	L	Cerebellum - Pyramis		1515	5.62	-12 -76 -23
E1			None				
E2			None				
LH			None				

[a] As for the main analyses, a combined voxel and clusterwise threshold was used to account for multiple comparisons (i.e., minimum 47 voxels for the ALFF analyses for $p<.05$, corrected). However, because the two cerebellar findings may be of interest in light of explaining some of the main findings, they have also been reported here although their conventional p-value would amount to $p=.08$, and $p=.11$ after correction, respectively.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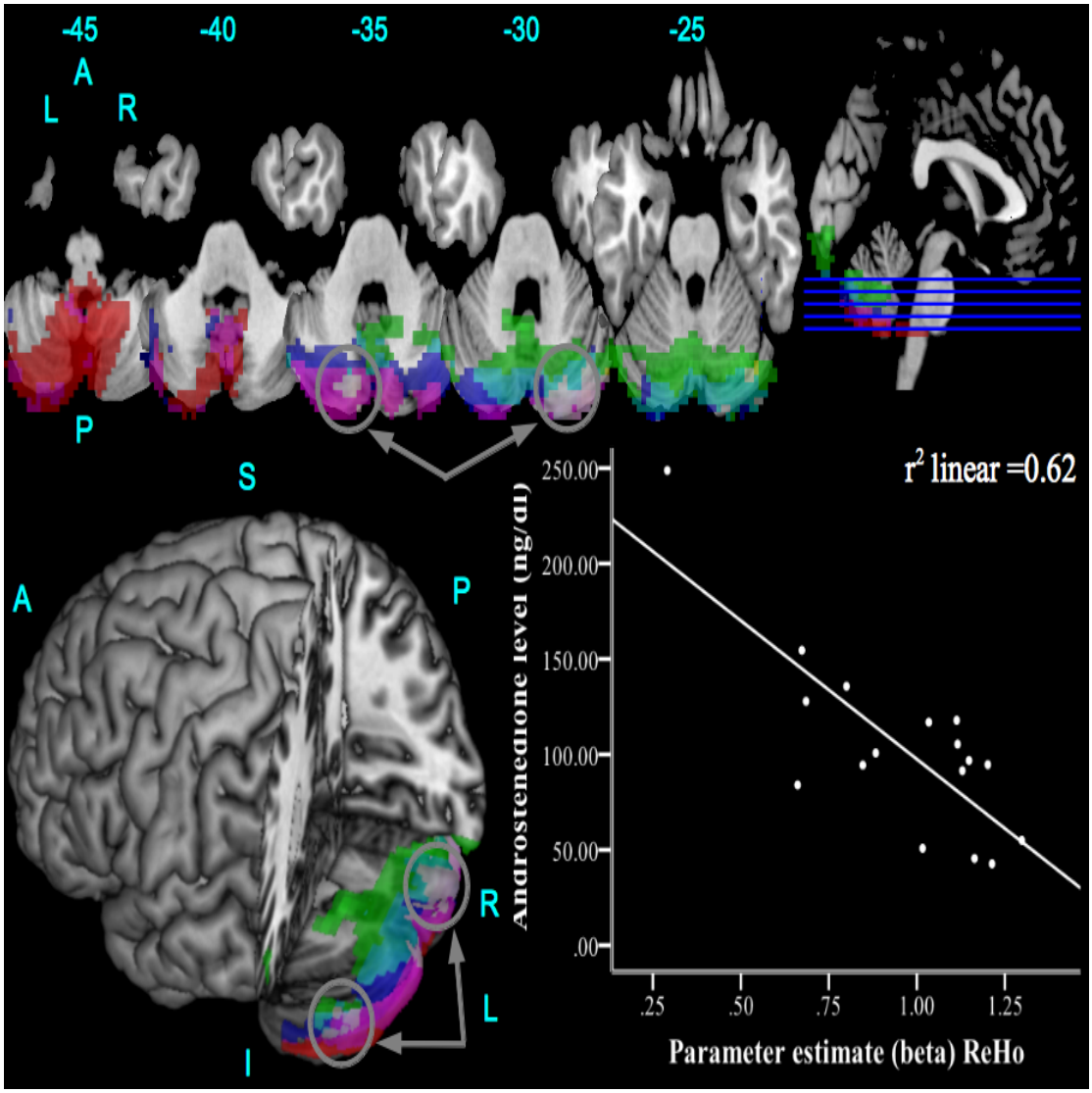
-45
-40
-35
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A
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S
A
P
R
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I
r² linear =0.62
Androstenedione level (ng/dl)
250.00
200.00
150.00
100.00
50.00
.00
.25
.50
.75
1.00
1.25
Parameter estimate (beta) ReHo