

Oestrogens are Not Related to Emotional Processing: a Study of Regional Brain Activity in Female-to-Male Transsexuals Under Gonadal Suppression

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Although the prevailing opinion is that emotional processes are influenced by sex hormones, the literature is still inconclusive. The aim of the current study was to examine the effects of gonadal suppression on brain activity during affective picture processing. Twenty-one female-to-male (FtM) transsexuals and 19 control women were recruited and underwent functional magnetic resonance imaging scanning while rating emotional pictures adapted from the International Affective Picture System. The gonadal hormone production of the FtMs was suppressed for 8 weeks, the control group did not receive any treatment before scanning. Under gonadal suppression, FtMs showed less brain activation in the superior temporal lobe compared with female controls during perception of positive affective pictures. Regression analysis showed that during processing of positive affective images, brain activity within the right superior temporal lobe was not correlated with levels of estradiol, luteinizing hormone, and follicle-stimulating hormone. In the absence of associations with hormonal levels, the difference in activation in the superior temporal lobe during positive emotional stimuli between FtMs and control women may be attributed to a priori differences between the 2 groups. Future studies should clarify if these differences are a result of atypical sexual differentiation of the brain in FtMs.

Keywords: emotional processing, functional MRI, gender dysphoria, gender identity disorder, GnRH suppression, sex steroids

Introduction

Sex steroids have organizational and activational effects on the brain (Phoenix et al. 1959). Organizational effects reflect the influence of hormones on the development (and sexual differentiation) of the brain, whereas activational effects refer to modulation of existing brain processes. Both mechanisms are likely to have a major role in behavioral development and differences between men and women, for instance in emotion processing. Females seem to be more reactive to emotional stimuli (Grossman and Wood 1993; Lang et al. 1993; Kring and Gordon 1998; Bradley et al. 2001; Kemp et al. 2004) and more emotionally expressive than males (Brody and Hall 1993). In addition, women are more accurate in decoding emotional facial expressions (Thayer and Johnsen 2000; McClure et al. 2004; Proverbio et al. 2006), appear to be more empathic than men when witnessing suffering humans (Singer et al. 2004; Schulte-Ruther et al. 2007; Han et al. 2008), and have better memory for emotional stimuli than men (Fujita et al. 1991; Seidlitz and Diener 1998).

The International Affective Picture System (IAPS) is frequently used in emotion research to enable researchers to induce emotions with a standardized set of pictures (Lang et al. 2008). Every IAPS picture has been rated with respect to both their valence category (from negative/aversive to positive/attractive) and arousal level (from a low level of activation/low intensity of the emotional reaction to a high level of activation/high intensity of the emotional reaction) on a nine-point scale.

Over the years, multiple functional magnetic resonance imaging (fMRI) studies using these IAPS items have reported sex differences in emotional responding (Lang et al. 1998; Wrase et al. 2003; Sabatinelli et al. 2004; Hofer et al. 2006; Caseras et al. 2007; Mak et al. 2009). Sex differences in brain activity were mostly found in frontal regions, especially the (ventrolateral) prefrontal cortex (Wrase et al. 2003; Hofer et al. 2006). Additionally, sex differences have been reported in the cingulate cortex, the cerebellum, the fusiform gyri, the insula, superior temporal gyrus, and the amygdala (Wrase et al. 2003; Hofer et al. 2006; Proverbio et al. 2006). In an fMRI study performed by Mak et al. (2009), men recruited a larger network during negative emotion regulation than women while viewing highly negative pictures. This lateral prefrontal network is commonly associated with cognitive processing, so that reduced activity in women could explain why women are generally more emotionally reactive to negative stimuli.

The effect of hormonal fluctuations on processing of emotional stimuli has rarely been studied. Goldstein et al. compared processing of negative emotional pictures during 2 periods (follicular and midcycle) of the menstrual cycle and found less activation in the amygdala, hypothalamus, hippocampus, and orbitofrontal cortex (OFC) during the midcycle timing compared with the early follicular phase. Since the amount of estradiol is low in the early follicular phase and much higher in the midcycle phase, these authors proposed that estradiol may induce a blunted response to negative stimuli in these brain areas (Goldstein et al. 2005). In 2010, Goldstein et al. performed a similar fMRI study, comparing signal intensity changes in specific regions of interest (ROIs) in men versus women in the early follicular phase, and in men versus women in the late follicular/midcycle phase. Results showed that the stress response circuitry activated in males was more similar to that of early follicular females than midcycle females, suggesting that activation differences were due to altered estrogen levels (Goldstein et al. 2010). To examine the effect of progesterone on affective processing, Andreano

et al. scanned women during the early follicular phase (low progesterone) and the midluteal phase (high progesterone) while viewing negative or neutral IAPS pictures. Increased reactivity of the amygdala and the hippocampus in response to negative stimuli was found during the midluteal phase. As progesterone levels but not estradiol levels differ significantly between the early follicular and midluteal phases, the increased reactivity of the amygdala and hippocampus was considered to be associated with the level of progesterone (Andreano and Cahill 2010). In contrast to the attenuating effect of estrogens (Goldstein et al. 2005, 2010), progesterone may increase activation of arousal circuitry during negative emotion processing. Andreano et al. therefore suggested that, from an evolutionary perspective, it may have been helpful for women to have a higher social sensitivity during their early follicular phase since it may facilitate social interaction and thereby mating behavior (Andreano and Cahill 2010). Thus, although the literature does not show wholly consistent results, estrogens, progesterone, or even the estrogens/progesterone ratio may have a role in activating or blunting neural activation during emotional processing. In this study, we will investigate the specific effect of gonadal hormone suppression on emotional processing with the use of a clinical model.

Individuals with gender identity disorder (GID) (American Psychiatric Association 2000) represent an interesting model to investigate these effects because of the unique hormonal treatment, whereas they show no endocrinological differences with other members of their natal sex before treatment (Gooren 1984). According to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV-TR) of the American Psychiatric Association, individuals with GID have a strong and persistent cross-gender identification, accompanied by persistent discomfort with their biological sex or sense of inappropriateness in the gender role of that sex (American Psychiatric Association 2000). Although the DSM-5 was published during the preparation of the manuscript, we will use DSM-IV-TR terminology and criteria, because the participants in our study were diagnosed according to DSM-IV-TR criteria.

In the present study, female-to-male transsexuals (FtMs) with a complete suppression of gonadal hormone production and control women without suppression performed an IAPS task during functional MRI scanning. Based on previous studies on female hormonal cycle effects on IAPS responses, we hypothesized to find an increase in brain activation in FtMs (suppression of estrogens) when compared with controls, in subcortical brain areas involved in emotional processes, such as the amygdala, hippocampus, and ventral striatum (Goldstein et al. 2005, 2010; Andreano and Cahill 2010). The amygdala and hippocampus are both important for emotional learning, the amygdala is a key structure involved in generating emotions, the hippocampus in the retrieval of memories for emotional events. Furthermore, within the group, gonadally suppressed FtMs, we also expected less brain activation in cortical areas such as the superior temporal lobe and OFC (Goldstein et al. 2005).

Materials and Methods

Subjects

Two groups of participants were investigated to study the relationship between estradiol and neuronal correlates of emotional processes. The

first group consisted of natal females with a GID, described here as FtMs. These individuals were tested after suppression of gonadotrophins with the use of GnRH analogs, and were compared with a control group of women without GID, with a regular and natural menstrual cycle. After providing written informed consent, 21 FtMs and 19 control women were enrolled in the study. The FtMs were recruited at the VU University Medical Center Amsterdam, the Netherlands, after receiving a diagnosis of GID (Doorn et al. 1994; Nieder et al. 2011). GID was diagnosed by psychologists according to DSM-IV-TR (American Psychiatric Association 2000) criteria. For more information on the diagnosis and treatment of people with GID at the VU University Medical Center in Amsterdam, see Kreukels et al. (2012). All FtMs reported an early onset (before puberty) of gender dysphoria. FtMs received hormonal treatment with a GnRH analog (triptoreline, 3.75 mg/4 weeks, subcutaneously) for 8 weeks to suppress gonadal hormone production. Due to continuous stimulation of the pituitary gland, the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) decreases, resulting in inhibition of gonadal hormone production.

The controls were recruited at the campus of the VU University in Amsterdam by flyers. They had a regular menstrual cycle of no longer than 35 days. None of the controls were allowed to use any hormonal contraceptives for the last 3 months. Participants were excluded from the study if they had ever received any kind of hormonal sex steroid treatment, other than contraceptives. To control for the effects of hormonal fluctuations during the menstrual cycle, we examined all control participants in the first 5 days of the menstrual cycle (follicular phase) to avoid progestogenic activity. Participants (controls and FtMs) with psychiatric, neurological, or endocrine disorders were excluded from the study.

The study was approved by the medical ethics committee of the VU University Medical Center Amsterdam, the Netherlands.

Procedure

During a test session, participants first completed a structured interview to obtain information regarding age, education, use of medication, and medical history. The session continued with a physical examination, after which blood samples were collected. Next, participants underwent a neuropsychological assessment (NPA) of ~1 h. At the end of the NPA session, a number of questionnaires were administered. The results of the physical examination and NPA session will be published elsewhere.

Before the fMRI scan, participants received task instructions outside the scanner. Subsequently, the participants underwent MRI scanning. The MRI scan session took 1 h to complete.

Instruments

The IAPS (Lang et al. 2008) is a collection of images that is often used in research to induce emotional processes. A selection of these images was successively presented via a screen. The task consisted of 30 positive valence images, 30 negative valence images, and 30 control images. Participants were asked to rate images as pleasant or unpleasant with 2 button boxes: the left button box for unpleasant and the right for pleasant ratings. Each image was presented for 2 s with a variable interstimulus interval (ISI) between 2.5 and 3.5 s. As control condition scrambled images were displayed with an arrow in the middle. In this control condition, participants were asked to indicate the direction in which the arrow was pointing.

To estimate the level of IQ participants had to complete the Dutch version of the National Adult Reading Test (NART) (Nelson and Wilson 1991). The test is commonly used to estimate premorbid level of IQ in patients with degenerative disorders or to provide an estimated level of IQ without having to administer a complete, but time consuming, test battery like the Wechsler Adult Intelligence scale.

This task consists of 50 words with atypical phonetic pronunciation. Subjects are required to read each word aloud.

For screening of psychopathology the Symptom Checklist-90-R (SCL-90-R) was used (Derogatis 1994). This questionnaire assesses self-reported psychological distress on 8 symptom scales: Somatization, Obsessive-Compulsive, Interpersonal Sensitivity, Depression, Anxiety,

Hostility, Phobic Anxiety, Paranoid Ideation, and Psychoticism. To assess general distress an overall scale, the Global Severity Index (GSI), was administered. Only the GSI, Anxiety, and Depression scales were used for this study.

MRI Acquisition

Imaging acquisition was performed on a whole-body 3T MR (Signa HDXt, General Electric, Milwaukee, WI, USA), located at the VU University Medical Center in Amsterdam, with the use of an SENSE-8 channel head coil. During the task, echo-planar images (EPIs) were obtained using axial T_2^* -weighted gradient-echo sequence. Repetition time (TR) = 2100 ms, echo time (TE) = 30 ms, matrix size = 96×96 , number of slices = 40, with a sequential ascending slice order.

Anatomical imaging included a sagittal 3D gradient-echo T_1 -weighted sequence. The settings used for anatomical imaging were: TR = 7.8 s, TE = 3.0 ms, matrix size = 256×256 , voxel size = $1 \times 1 \times 1$ mm, and number of slices = 170.

Hormonal Assays

From the collected blood samples, serum levels of several hormones were monitored. Estradiol was measured by a competitive fluorescence immunoassay (PerkinElmer, Wallac Turku, Finland) with a lower limit of detection of 20 pmol/L and an interassay coefficient of variation (CV) of <10%. For testosterone and DHEA-S a radioimmunoassay (Siemens Medical Solutions Diagnostics, USA) was used with a lower limit of detection of 1 nmol/L and an interassay CV of <7%, and a lower limit of detection of 0.2 μ mol/L and an interassay CV of <9%, respectively. Both LH and FSH were measured by commercially available fluorescence immunometric assays (Abbott Laboratories, IL, USA). The lower limit of detection was 2 nmol/L for both LH and FSH. Progesterone was measured with a competitive fluorescence immunoassay (Abbott Laboratories) with a lower limit detection of 2 nmol/L and an interassay CV of <5%. The levels of sex hormone-binding globulin and prolactin were measured with a fluorescence immunometric assay (Siemens Medical Solutions Diagnostics) with a lower limit of detection of 2 nmol/L and an interassay CV of <4%, and a lower limit of detection of 0.05 U/L and an interassay CV of <6%, respectively. Androstenedione was measured by radioimmunoassay (Webster, TX, USA) with a lower limit of detection of 0.5 nmol/L and an interassay CV of <9%. Finally, thyroid-stimulating hormone (TSH) was measured with an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) with a lower limit of detection of 0.005 mU/L and an interassay CV of <3%.

Statistical Analysis

Statistical Parametric Mapping 8 (SPM8) (<http://www.fil.ion.ucl.ac.uk/spm>), and a standard software suite of MATLAB (the Mathworks, Inc., Natick, MA, USA), was used for the fMRI analysis. Images were manually reoriented and the origin was set to the anterior commissure. Slice timing was used to correct for differences in acquisition time, followed by realignment to correct for possible movements in the scanner. The EPIs were co-registered to the T_1 anatomical image, which was segmented and normalized to a standard brain space defined by the Montreal Neurological Institute. The preprocessing steps were finalized with an 8-mm full-width half-maximum Gaussian smoothing filter.

First-level analyses were performed for each subject, during which contrast images were computed for positive and negative stimuli versus control condition. These contrast images were used for second-level (group) analyses.

A between-group analysis was carried out for each group and condition (positive affective images and negative images condition) with the use of a full-factorial design to evaluate main ($P < 0.05$, whole-brain family-wise error corrected and interaction effects ($P < 0.05$, small-volume corrected) for a priori defined cortical and subcortical brain areas: superior temporal lobe and orbitofrontal lobe, amygdala, hippocampus, insula, and ventral striatum. All ROIs were defined with the use of a predefined automatic anatomical labeling atlas.

To examine the relationship between estradiol, LH, and FSH, and brain activity, and to calculate the individual contribution of these

hormones to the BOLD response, regression analyses were performed with SPM8. The hormone levels of estradiol, LH, and FSH were defined as predictors and BOLD response as dependent variable.

Questionnaire, sociodemographic and performance data, and hormonal assessments were analyzed with the Statistical Package for the Social Sciences, version 20. Independent sample t -tests and analyses of variance were used to analyze between-group differences. When the assumption of homogeneity of variance was violated, groups were compared with nonparametric Kruskal–Wallis tests and post hoc tested with Mann–Whitney U -tests.

Results

Sample

Forty-four participants were approached of whom 40 could participate. Four participants (all FtMs) did not agree to take part in the study because participating in the study would delay their regular medical care. Baseline characteristics of cases and controls are summarized in Table 1. The mean age of the FtMs was significantly lower than the controls ($P = 0.01$, see Table 1). The groups did not differ in IQ estimate (NART) (Table 1).

The GSI of the SCL-90-R differed significantly between the 2 groups. The FtMs had a higher score on this general distress measure than the control group ($P = 0.02$); however, this score was still relatively low and gave no indication for clinically relevant psychological distress in the FtMs under study (Pedersen and Karterud 2004). No group differences were found in Anxiety and Depression subscales of the SCL-90 (Table 2).

Hormonal Levels

Suppression of the gonadal steroids in the FtM group was confirmed by the measurements of hormonal levels. In FtMs the levels of LH, FSH, and estradiol were significantly lower than in controls ($P < 0.001$). None of the other hormonal levels showed a significant difference between the groups (Table 2).

International Affective Picture System

Performance Data

During the IAPS task, no differences in reaction time were found between the groups for the 3 conditions (Table 3).

fMRI Data

Main effect for emotional processing. Performance of the IAPS task was associated for both groups with bilateral activation in the dorsolateral and dorsomedial prefrontal cortex and superior temporal lobe. Furthermore, brain activation was found in the cerebellum. Within the limbic system, the thalamus, amygdala, and hippocampus showed activation (Table 4).

Table 1
Demographic information

	FtMs ($N = 21$)	Controls ($N = 19$)	P -value
Age (SD)	22.6 (6.9)	25.8 (6.3)	0.01*
Range	17.5–43.3	18.8–42.7	
Estimated IQ (SD)	97.7(14.3)	102.6 (9.3)	0.2
Range	71–126	93–126	

*The groups differed significantly ($P < 0.05$) with regard to age. The assumption of homogeneity of variance was violated; therefore, variables were tested with nonparametric tests. Post hoc tests with Mann–Whitney U -showed significant differences between the 2 groups ($U = 104.00$, $z = -2.59$, $P = 0.01$).

Table 2

Symptom checklist 90-revised scores and hormonal levels

	FtMs (N = 21)	Controls (N = 19)	P-value
Symptom checklist 90-revised			
Global Severity Index (SD) ^a	0.34 (0.2)	0.21 (0.15)	0.02*
Range	0.06–0.77	0.07–0.63	
Depression Scale (SD)	0.34 (0.05)	0.25 (0.05)	0.23
Range	0.00–0.81	0.00–0.63	
Anxiety scale (SD)	0.26 (0.05)	0.16 (0.03)	0.13
Range	0.00–0.70	0.00–0.40	
Hormonal levels (SD)			
A'dion	4.9 (2.8)	6.1 (2.9)	0.07
DHEA-S	5.9 (2.1)	4.8 (1.9)	0.21
LH	0.3 (0.2)	4.0 (1.6)	<0.001*
FSH	1.8 (1.1)	5.6 (1.4)	<0.001*
PRL	0.18 (0.1)	0.19 (0.1)	0.83
P	<2.0	<2.0	N/A
E2	33.4 (12.8)	133.9 (48.5)	<0.001*
T	1.1 (0.24)	1.1 (0.2)	0.91
SHBG	41.3 (14.8)	48.5 (13.8)	0.16
TSH	2.1 (0.9)	2.2 (1.3)	0.67

Note: A'dion, androstenedione in nmol/L; DHEA-S, dehydroepiandrosterone in μ mol/L; LH, luteinizing hormone in U/L; FSH, follicle-stimulating hormone in U/L; PRL, prolactin in U/L; P, progesterone in nmol/L; E2, estradiol in pmol/L; T, testosterone in nmol/L; SHBG, sex hormone-binding globulin in nmol/L; TSH in mU/L.

^aFor the Global Severity Index (GSI): FtMs ($n = 20$) and Controls ($n = 18$).

*The groups differed significantly ($P < 0.05$) with regard to Global severity index. The assumption of homogeneity of variance was violated; therefore, variables were tested with nonparametric tests. Post hoc tests with Mann-Whitney U -test showed significant differences between the 2 groups ($U = 98.00, z = -2.40, P = 0.02$). Furthermore, there was a significant difference in the levels of estradiol, LH, and FSH. Estradiol ($U = 1.00, z = -5.382, P < 0.01$), LH ($U < 0.01$), $z = -5.41, P < 0.01$), and FSH ($U = 6.50, z = -5.41, P < 0.01$).

Table 3

Reaction time in milliseconds to positive and negative images on the IAPS task

	FtMs (N = 21)	Controls (N = 19)	P-value
Positive affective images	1012 (101)	972 (124)	0.32
Range	857–1236	789–1180	
Negative affective images	1009 (111)	969 (133)	0.20
Range	774–1170	690–1232	
Control images	631 (117)	619 (57)	0.85
Range	522–1097	522–722	

Note: The assumption of homogeneity of variance was violated; therefore, variables were tested with nonparametric tests.

Group comparisons

Positive affective images. While processing positive affective images, activation was found in the right superior temporal lobe in both groups, but the FtMs showed less activation compared with the controls (Fig. 1 and Table 4).

Regression analysis showed that, during processing of positive affective images, brain activity within the right superior temporal lobe was not significantly correlated with estradiol, LH, or FSH levels. Although there were no other differences in brain activity between the 2 groups, activation in other brain areas outside the ROI did correlate with the levels of estrogens, LH, and FSH. The results are described in Table 4. A regression analysis was also performed with age as a predictor, but did not reveal any significant associations.

Negative affective images. While processing negative affective images the FtMs and control group did not differ significantly in brain activity in our ROIs.

Discussion

In the current study, FtMs under gonadal suppression and controls (women with an unaltered hormone production)

Table 4

Brain areas (main effect and group differences) during the positive and negative condition of the IAPS

Brain region	MNI coordinates				Number of voxels
	X	y	z	t	
Main effect					
Dorsomedial prefrontal cortex	–3	59	22	12.50	1116
Dorsolateral prefrontal cortex L.	–42	20	–11	13.01	711
Dorsolateral prefrontal cortex R.	54	32	7	10.28	535
Superior temporal lobe R.	42	17	–20	7.58	29
Superior temporal lobe L.	–45	14	–17	10.82	40
Amygdala L.	27	–4	–20	12.30	37
Amygdala R.	–24	–7	–17	11.91	18
Hippocampus L.	–30	–10	–20	15.44	171
Hippocampus R.	27	–7	–20	12.69	174
Thalamus L.	–9	–7	4	7.33	146
Cerebellum	30	–46	–20	17.16	727
Difference positive affective images					
Controls > FtMs					
Temporal superior R.	39	11	–23	3.29	16
Negative correlate with LH					
Cingulum ant. R.	3	47	19	3.51	233
Frontal superior L.	–15	32	34	3.68	44
Precentral R.	57	2	25	3.67	32
Heschl R.	51	–13	4	3.24	33
Temporal mid R.	48	–46	7	3.65	63
Precuneus	3	–52	25	4.90	131
Positive correlate with FSH					
Frontal superior L.	–18	29	–31	4.16	32
Difference negative affective images					
Controls > FtMs					
Frontal mid R.	33	47	25	3.27	12
Controls > FtMs					
Cingulum ant.	–3	41	4	3.89	48
Precuneus R.	3	–55	25	3.63	117
Negative correlate with LH					
Frontal superior medial R.	3	50	4	3.42	135
Cingulum ant.	""	""	""	""	""
Temporal mid R.	51	–43	7	4.51	117
Temporal mid L.	–54	–55	1	3.41	38

Note: Within the activation map both regions (the frontal superior medial Right and Cingulum ant.) are connected with each other. The quotes were used to emphasize that the previous reported area included the Cingulum ant.

performed an emotional picture processing task in an MRI scanner. Because we were interested in the role of estrogens in emotional processing, our aim was to study if gonadal suppression, and hence lower estradiol levels, were associated with altered brain activation during emotional processing. FtMs under gonadal suppression had indeed significantly lower levels of estradiol than the control women. During processing of positive affective images, the right superior temporal lobe was activated in both groups, but the FtMs showed less activation in this brain area compared with the controls. However, this difference was unrelated to hormonal levels.

Differences in the superior temporal lobe have also been found in studies that investigated sex differences during emotional processing. In an EEG study, Proverbio et al. (2009) found stronger superior temporal gyrus activation in women than in men when viewing negative pictures. Hofer et al. (2006) demonstrated that women showed greater activation of several brain areas, including the right superior temporal lobe compared with men when viewing negative pictures.

The superior temporal lobe is involved in several cognitive processes as well as emotion processing and social cognition, as several studies have implicated the superior temporal lobe in processing social stimuli (Skuse et al. 2003; Pelphrey et al. 2004). Regarding the various components of social cognition (Adolphs 2001), the superior temporal sulcus has been

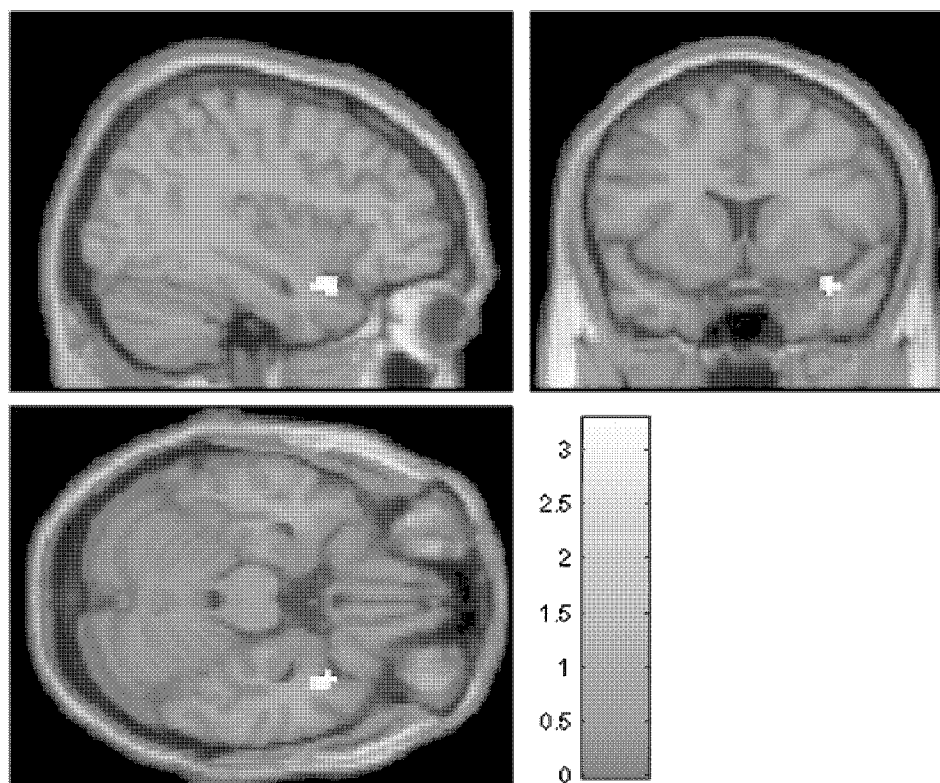


Figure 1. Brain areas with increased activation in the controls compared with FtMs during positive condition of the IAPS.

described as an area that perceives social stimuli such as faces, gaze, and mouth movements (Puce et al. 1998).

It is assumed that a variety of hormones have a role in sex differences in emotional perception and experience (Goldstein et al. 2005, 2010; Andreano and Cahill 2010). The general view is that estrogens have an inhibiting effect on the arousal system as was found in the study by Goldstein et al. (2005) who reported a blunted amygdala response in the menstrual cycle phase with higher estrogen levels. Thus, higher estrogen levels, particularly present during the follicular phase, are associated with lower arousal. Goldstein et al. (2010) proposed that, from an evolutionary perspective, this lowered arousal may have helped females in making judgments about whether a situation may support successful mating by not being distracted by excessive arousal. Especially during the follicular phase, such judgments can be of importance (Goldstein et al. 2010).

In our study, the groups did not differ in brain activity in limbic regions, but they did in cortical areas. The FtMs who were gonadally suppressed showed less activation of the superior temporal lobe than the control women during processing of positive affective images. This cortical area may have an inhibiting effect on limbic structures as suggested by Goldstein et al. (2010), which effect may be due to changes in perception of stimuli. This is in accordance with the ideas of LeDoux (2000) who described a cortical control system of the amygdala: Higher estrogen levels may enhance activation of cortical structures, resulting in greater cortical control which in turn may lead to lowered activation of the amygdala (LeDoux 2000). However, our data do not clearly support such a role of estrogen nor the inhibiting role of cortical structures on limbic structures.

Besides the difference in estrogen levels between the groups, LH and FSH levels also differed. This is a direct result of the administration of the GnRH analog in the FtM group. The hypothalamic–pituitary–ovarian axis functions as a feedback system and the levels of LH, FSH, and estrogens are inter-related, so that disentangling the effects of these 3 hormones on brain activity is not straightforward. A positron emission tomography study investigating modulation of neuronal activity by gonadal steroid hormones in young women during an executive functioning task showed changes in regional blood flow (rCBF) during GnRH analog downregulation with the use of a GnRH analog (Berman et al. 1997). This change in rCBF was restored after administration of estradiol or progesterone. In our study, we found a negative relation between activation patterns in the anterior cingulate cortex, superior frontal lobe, midpart of the temporal lobe, precentralis Heschl gyrus and precuneus and, the levels of LH during the processing of positive affective images. It may thus be that LH rather than estrogens affects emotional processing. Whether LH affects emotional processing independent of FSH and E warrants further study, as this could not be studied within the current design.

Age differed between the 2 groups; control women were older than FtMs. However, a regression analysis showed no significant associations with task-related BOLD response; therefore, it is unlikely that age affected the results.

In the current study, we investigated a group of natal women with GID and compared them with women without GID. Previous studies did show several structural and functional brain differences between individuals with GID and members of their natal sex. Differences were found in FtMs

versus control women with regard to white matter microstructure (Rametti et al. 2011), subcortical volume (putamen) (Zubiaurre-Elorza et al. 2013), and rCBF in the insula and ACC (Nawata et al. 2010). However, not all studies investigating a priori differences in brain structure or functionality support this observation. Santarnecchi et al. (2012) investigated brain functionality with the use of resting-state analysis. One untreated FtM was compared with group of female and male control subjects. The connectivity profile of the FtM was closer to the biological sex than to the desired sex. To our knowledge, brain activation during emotional processing has not been studied previously in FtMs. It may be that the FtMs were already different from the female control group before the start of GnRH analogs and that the observed group differences were due to prenatal hormonal influences rather than circulating hormones. Future studies may include a drug naïve FtM group, or perform baseline measurements before the start of GnRH analogs to study a priori differences between the groups. To control for differences between the groups with regard to psychological symptoms, we administered the SCL-90. None of the subscales, including dimensions that may affect emotional processes such as depression and anxiety, were found to differ between the groups.

Because differences in personality have been reported to affect neural activation during emotional processing (Calder et al. 2011) and differences in temperament and personality traits between transsexuals and controls have been described (Gomez-Gil et al. 2013), future research may also benefit from including a questionnaire on personality. Nevertheless, the absence of an association between hormonal levels and brain activation during positive emotional stimuli in the superior temporal lobe (STL) makes it unlikely that the group differences were related to the differences in circulating hormones between the groups. In previous studies, natal men, like our group of gender dysphoric women, also showed lower activation in the STL compared with nongender dysphoric natal women (Hofer et al. 2006; Proverbio et al. 2009). It is therefore possible that our finding is a result of prenatal atypical sexual differentiation of the brain in FtMs. To test this hypothesis, a future study should include a baseline measurement before the start of GnRH analogs, to test if the groups were already different before the start of gonadal suppression. This study did not find associations between levels of estrogens and differences in brain activity during emotional processing. Therefore, the role of circulating levels of estrogens in emotional processing is still debatable. More fMRI studies are needed to examine the influence of specific hormones, in particular estrogens, on emotional processing.

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