ORIGINAL RESEARCH—INTERSEX AND GENDER IDENTITY DISORDERS

Sex Differences in Verbal Fluency during Adolescence: A Functional Magnetic Resonance Imaging Study in Gender Dysphoric and Control Boys and Girls

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ABSTRACT

Introduction. In the literature, verbal fluency (VF) is generally described as a female-favoring task. Although it is conceivable that this sex difference only evolves during adolescence or adulthood under influence of sex steroids, this has never been investigated in young adolescents.

Aim. First, to assess sex differences in VF performance and regional brain activation in adolescents. Second, to determine if untreated transsexual adolescents differ from their sex of birth with regard to VF performance and regional brain activation.

Method. Twenty-five boys, 26 girls, 8 Male-to-Female transsexual adolescents (MtFs), and 14 Female-to-Male transsexual adolescents (FtMs) were tested in a cross-sectional study, while performing a phonetic and semantic VF task within an MRI scanner.

Main Outcome Measures. Functional MRI response during VF task.

Results. Boys and girls produced similar amounts of words, but the group MtFs produced significantly more words in the phonetic condition compared to control boys, girls, and FtMs. During the semantic condition, no differences were found. With regard to brain activity, control boys showed more activation in the right Rolandic operculum, a small area adjacent to Broca's area, compared to girls. No significant differences in brain activity were found comparing transsexual adolescents, although sub-threshold activation was found in the right Rolandic operculum indicating a trendwise increase in activation from control girls to FtMs to MtFs to control boys.

Conclusions. The better performance of MtFs is consistent with our expectation that MtFs perform better on female-favoring tasks. Moreover, they produced more words than girls and FtMs. Even though a trendwise linear increase in brain activity between the four groups only approached significance, it may indicate differences in individuals with gender identity disorder compared to their birth sex. Although our findings should thus be interpreted with caution, they suggest a biological basis for both transgender groups performing in-between the two sexes. **Soleman RS, Schagen SEE, Veltman DJ, Kreukels BPC, Cohen-Kettenis PT, Lambalk CB, Wouters F, and Delemarre-van de Waal HA. Sex differences in verbal fluency during adolescence: A functional magnetic resonance imaging study in gender dysphoric and control boys and girls. J Sex Med 2013;10:1969–1977.**

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Introduction

Gender dysphoria refers to the distress resulting from incongruence between experienced/expressed and assigned gender. The diagnosis gender identity disorder (GID) in the *Diagnostic and Statistical Manual of Mental Disorders–IV-TR* (*DSM–IV-TR*) [1]; or transsexualism [2] is an extreme form of gender dysphoria, characterized by strong and persistent cross-gender identification, accompanied by persistent discomfort with the biological sex or sense of inappropriateness in the gender role of that sex.

In a number of studies, potential determinants of GID have been investigated. In post-mortem brain material of the hypothalamus, similarities in volume and neuron number of the bed nucleus of the stria terminalis (BSTc) [3,4] and the interstitial nucleus of the anterior hypothalamus (INAH-3) [5] were found in male-to-female (MtF) transsexuals and control women, whereas these nuclei differed from control men. These results suggest that GID-related differences in the brain may result from an altered sensitivity to prenatal exposure of sex steroids and may underlie atypical gender identity development. This hypothesis is in line with some neuropsychological studies showing that transsexuals perform in the direction of their experienced rather than their assigned gender on sex-specific tasks [6,7]. Cohen-Kettenis et al. [6] showed in 1998 that MtFs performed better than males during a female-favored verbal memory task, although not all confirm this hypothesis [8]. Haraldsen et al. [8] found cognitive patterns consistent with their biological sex instead of their gender identity. A number of imaging studies to date have provided evidence for brain differences between transsexuals and non-transsexual controls of their sex of birth, as well as brain similarities in the brain of transsexuals with controls of their desired gender with regard to white matter microstructure [9], shape of the corpus callosum [10], grey matter of the putamen [11], hypothalamic activation in response to odorous steroids [12], cerebral activation during visual erotic stimuli [13], and mental rotation [14]. One imaging study failed to detect brain differences between transsexuals and controls [15].

In general, women tend to outperform men with regard to verbal abilities [16]. Although there are various neuropsychological tasks to investigate this domain, the verbal fluency (VF) task, also known as the controlled word association test (COWAT), is most frequently used [17,18]. Subjects have to produce as many words as possible in 60 seconds, beginning with a particular letter (phonetic fluency) or belonging to a specific category (semantic fluency). Women generally produce more words [19,20] and tend to use different strategies during VF than men. Lanting et al. [21] describes how participants use clusters as strategy to produce as many words as possible and found that males produce more words within a particular cluster before switching. This effect was found for both phonetic and semantic fluency. Females were found to switch more often between the clusters, but only during the semantic fluency condition [21]. Cognitive sex differences have not only been observed in adulthood. In the mid-1970s, Maccoby and Jacklin [16] concluded, based on an analysis of 85 studies, that females start to develop a superiority in verbal abilities around the age of 11.

Neuroimaging techniques such as functional magnetic resonance imaging (fMRI) have been employed to clarify the underlying neuronal mechanisms of sex differences in VF. In an fMRI study on VF, women generated more words and showed greater regional brain activity than men during phonetic fluency [22]. Weiss and colleagues found similar patterns of brain activation when only high-performing men and women were compared [23], characterized by activation in the left prefrontal cortex together with activation of the anterior cingulate and right cerebellum. In contrast, another study reported stronger activation in the left inferior temporal gyrus, anterior and posterior cingulate cortex, right anterior cingulate cortex, superior frontal gyrus, dorsolateral prefrontal cortex, and lingual gyrus in men compared to women during VF regardless of performance [24]. Based on these results, Gauthier et al. suggested that men use more visualization strategies during word generation to reach a similar level of VF performance as women [24].

To our knowledge, individuals with GID have never been studied using VF tasks and fMRI. In the literature, two forms of GID have been described: early onset and late onset GID [25,26], defined by an onset of gender dysphoria before puberty and during or after puberty, respectively. These groups differ with respect to a number of characteristics (e.g., developmental trajectory, role of sexual arousal, sexual orientation). The majority of imaging studies in transsexuals did not report on the onset age of GID, or investigated a heterogeneous group [11,14,27]. Therefore, previous inconsistent results may have been due to differences between studies with regard to the onset age of the subjects. The current study therefore included a homogeneous group of adolescents with GID with an early onset form of transsexualism.

The aim of the current study was twofold: (i) to assess sex differences in VF performance and regional brain activation during VF in adolescents; and (ii) to determine if untreated transsexual adolescents differ from their sex of birth with regard to VF performance and regional brain activation. If a female-favored task such as the VF is influenced by organizational effects of sex steroids, we expect differences in VF performance and brain activity between boys and girls during adolescence. Specifically, in line with the literature [22–24], we expect girls to outperform boys and to show less brain activity than boys within the previously described brain areas, including the anterior cingulate cortex and superior frontal gyrus, but also language-related areas such as the inferior frontal lobe (Broca's area) and superior temporal gyrus (Wernicke's area). We also expect MtFs to produce more words than control males, and activate brain areas more like control females [19,20]. We expected a similar effect in female-to-male transsexuals (FtMs), but in the opposite direction.

Methods

Subjects

Participants were recruited in a research program investigating puberty suppression with gonadotrophin releasing hormone analogues (GnRHa) and subsequent cross-sex hormone treatment, from the age of 16 years, in adolescents diagnosed with GID at the VU University Medical Center in Amsterdam [28] The present study is part of a larger research project with a focus on brain development and brain functioning in juvenile transsexuals before and during treatment with GnRH (Trial registration number ISRCTN 81574253, (http://www.controlled-trials.com/isrctn/). After providing written informed consent, 8 MtFs and 14 FtMs were enrolled in the study. Adolescents with GID were diagnosed by psychologists according to the criteria described in the *DSM–IV-TR* [29]. For additional information regarding diagnosis and treatment of adolescents with GID in our center, we refer to (Kreukels and Cohen-Kettenis) [28]. All individuals of the GID groups reported to be erotically attracted to the same natal sex (a homosexual orientation), except for one MtF who reported to be bisexual. The adolescents with GID were studied before they started GnRHa treatment. None of the participants had ever received any kind of hormonal treatment. For the age-matched control groups, friends and relatives of the participating adolescents with GID were invited: 25 control boys and 26 control girls. All controls had a heterosexual orientation.

Procedure

After arrival, participants underwent a physical examination to determine height, weight, blood pressure, and puberty stage according to Tanner [30]. Intelligence was then estimated with the use of the Wechsler Intelligence Scale for Children (WISC-III®) [31] or the Wechsler Adult Intelligence Scale (WAIS-III®) [32] depending on their age. Subsequently, all subjects underwent MRI scanning while performing four cognitive tasks, of which one was the VF task. Before MRI scanning, task instructions were given, and the tasks were practiced outside the scanner until they were completely understood. All four tasks were randomly presented during the scanning session. Data of the other tasks will be reported elsewhere. The scanning session took 1 hour to complete.

Task Paradigm

The VF task (based on Altena et al. [33]) consisted of three conditions: phonetic fluency, semantic fluency, and a control condition. In the phonetic fluency condition, participants were asked to generate as many words as possible beginning with a particular letter within 30 seconds. During the task, the letters K, O, M or D, A, T were presented successively via a screen. The two series (K, O, M and D, A, T) were chosen because of their high and similar frequency of occurrence in the Dutch language and their presentation was varied with a predefined order.

During the semantic fluency task condition, subjects were asked to produce as many words as possible belonging to a given category. Three categories—fruits and vegetables, clothes and toys, or animals, professions, and sports—were presented consecutively on the screen. Finally, the subjects were asked to count backwards for 15 seconds as a control condition. For every produced word or digit, the participant was asked to push a button. All conditions were presented in alternating order.

MRI Acquisition

Imaging acquisition was performed on a 3 Tesla Philips Intera (Best, the Netherlands) scanner with the use of a SENSE 6-channel head coil at the Academic Medical Center, Amsterdam, the Netherlands. During the verbal fluency test, echo-planar images (EPI) were obtained using an axial T2*-weighted gradient echo sequence. The settings for this sequence were: repetition time (TR) = 2,300 ms, echo time (TE) = 30 ms, matrix size = 96 × 96, number of slices = 35, and with an interleaved slice order.

Anatomical imaging included a sagittal three-dimensional gradient-echo T1-weighted sequence. The settings used for anatomical imaging were: TR = 9 ms, TE = 3.5 ms, matrix size = 256 × 256, voxel size = 1 × 1 × 1 mm, and number of slices = 170.

Statistical Analysis

Statistical Parametric Mapping 8 (SPM8) (http://www.fil.ion.ucl.ac.uk/spm), and a standard software suite of MATLAB (Mathworks, Inc., Natick, MA, USA), was used for the fMRI analyses. Images were manually reoriented and the origin was set to the anterior commissure. Slice timing was used to correct for differences in acquisition time and possible movements in the scanner were corrected with realignment to the first volume as reference. The echo planar images (EPI) were co-registered to a segmented anatomical image and normalized to a standard brain space defined by the Montreal Neurological Institute (MNI). The preprocessing steps were finalized with an 8 mm FWHM Gaussian smoothing filter.

For the first-level analyses, task conditions were modeled using boxcar regressors convolved with a canonical hemodynamic response function. Next, contrast images for phonetic fluency vs. control condition and semantic fluency vs. control condition were entered into second-level analyses of variance to assess main effects for task as well as group by task interaction effects. Main effects for the task are reported at $P < 0.05$ family wise error (FWE)-corrected and group by task interaction effects, within the *a priori* hypothesized brain areas at $P < 0.005$ uncorrected. The threshold for cluster size was set to 10 voxels. Neuropsychological and questionnaire data were scored according to standard scoring rules. VF performance data were analyzed with the Statistical Package for the Social Sciences (SPSS), version 19. Independent sample t-tests and analyses of variance were used for the analyses of between-group differences. However, if variance homogeneity assumptions were violated, the groups were compared with non-parametric Kruskal-Wallis tests and post hoc testing with Mann-Whitney U-tests. Statistical tests were considered to be significant at $P < 0.05$.

Results

Sample

Seventy-three adolescents were included in this study with a mean age of 14.5. In total, 6 adolescents (2 MtF, 3 FtM, 1 control boy) had to be excluded from the analysis, due to insufficient quality of the MRI scans. Eleven subjects were left-handed (1 MtF [13%], 2 FtM [14%], 4 control boys [16%], 4 control girls [15%]). No significant differences in the distribution of handedness, age, IQ, and Tanner stage were found between the groups (Table 1).

VF Performance Data

During the phonetic condition, MtF adolescents produced significantly more words than the other

Table 1 Sample characteristics

	MtFs (N = 8)	FtMs (N = 14)	Control boys (N = 25)	Control girls (N = 26)	*P*	
Age (years)						
Mean (SD)	14.25 (2.68)	14.64 (2.45)	14.68 (1.67)	14.44 (1.77)	0.832	NS
Range	11.55–19.54	11.64–18.26	11.67–16.68	11.59–17.54		
IQ						
Mean (SD)	118.50 (21.55)	97.93 (14.18)	109.24 (18.51)	101.54 (18.14)	0.054	NS
Range	89–143	81–131	89–143	71–133		
Tanner stage G						
Mean (SD)	3.75 (1.17)		3.92 (1.26)		0.726	NS
Range	2–5		2–5			
Tanner stage M						
Mean (SD)		4.36 (1.01)		4.31 (0.84)	0.664	NS
Range		2–5		2–5		
Handedness						
Right-handed (%)	7 (87.5%)	12 (85.7%)	21 (84%)	22 (84.6%)	1.00	NS
Left-handed (%)	1 (12.5%)	2 (14.3%)	4 (16.0%)	4 (15.4%)		

Abbreviations: MtF = male-to-female adolescents; FtM = female-to-male adolescents; SD = standard deviation

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Table 2 Number of words produced during phonetic and semantic fluency by group

	MtFs (N = 8)	FtMs (N = 14)	Control boys (N = 25)	Control girls (N = 26)	*P*	
Phonetic fluency						
Mean (SD)	37.37* (5.88)	28.50 (6.77)	30.32 (7.40)	31.60 (8.20)	0.031*	
Range	29–45	18–41	20–48	19–51		
Semantic fluency						
Mean (SD)	46.75 (11.07)	40.92 (9.25)	38.60 (9.39)	43.36 (11.30)	0.337	NS
Range	31–62	21–59	18–55	29–74		

*The groups differed significantly with regard to the number of produced words during phonetic fluency. Post-hoc tests with Mann-Whitney U showed significant differences between Boys and MtFs (U = 41.50, z = −2.46, $P = 0.012$), Girls and MtFs (U = 48.50, z = −2,17, $P = 0.028$), and MtFs and FtMs (U = 16.00, z = −2.74, $P = 0.005$).
Abbreviations: MtF = male-to-female adolescents; FtM = female-to-male adolescents; SD = standard deviation

groups (Table 2). In the semantic fluency condition, no significant differences in number of produced words were found between the groups.

fMRI Data

Main Effect

VF performance of the phonetic fluency task was associated with activation in the dorsolateral prefrontal cortex (DLPFC) (BA 45) extending to the right insula (BA 13) and right superior temporal lobe (BA 22). Furthermore, the anterior cingulate cortex (BA 24), thalamus and part of the occipital lobe were activated (Table 3). The semantic fluency condition showed a similar pattern, activation in the DLPFC, cingulated cortex and occipital lobe. However, during this condition there was no activation in the right superior temporal lobe, insula or thalamus (Table 3).

Group Effects: Phonetic Fluency

Between the two GID groups, we did not find activation differences at our *a priori* threshold. Control boys showed increased activation in the right Rolandic operculum (BA 43) compared to control girls (Figure 1 and Table 3). Post hoc analyses showed a trendwise increase of activation within the right Rolandic operculum across the four groups: the least activation was found in control girls followed by the FtMs, MtFs and the most activation in the control boys (Figure 2). No other significant differences were found between MtF, FtM, and the control groups.

Semantic Fluency

No significant differences were found in the semantic fluency condition between the single groups. However, there was trendwise linear activation between the groups within the left insula. The least activation was in the control girls–FtMs–MtFs and the most activation was in the group boys.

Table 3 Regions with significantly increased brain activity during the two different conditions. (All regions were significant at $P < 0.05$ FWE-corrected for main effects and $P < 0.005$, uncorrected for interaction effects.)

Brain region	BA	MNI coordinates x	y	z	t	*P*	Number of voxels
Main effect phonetic fluency							
Frontal inf. tri	45	−48	29	16	13.67	<0.001	5096
Cingulum ant.	24	−6	35	22	9,54	<0.001	
Superior occipital lobe left	NA	−18	−82	25	5.79	<0.001	
Insula right	13	39	23	−5	7.14	<0.001	232
Thalamus right	NA	9	−13	−2	5.50	<0.001	14
Temporal sup. right	22	54	−13	1	5.06	<0.001	10
Main effect semantic fluency							
Frontal inf. tri. right	45	−48	23	22	10.4	<0.001	651
Cingulum ant.	24	−3	23	22	9.29	<0.001	467
Lingual gyrus left	18	−12	−55	1	8.12	<0.001	419
Frontal mid. left	6	−30	11	52	6.74	<0.001	60
Phonetic fluency (Increased activation in control boys versus control girls)							
Rolandic operculum	4/43	54	−22	19	2.95	0.002	15

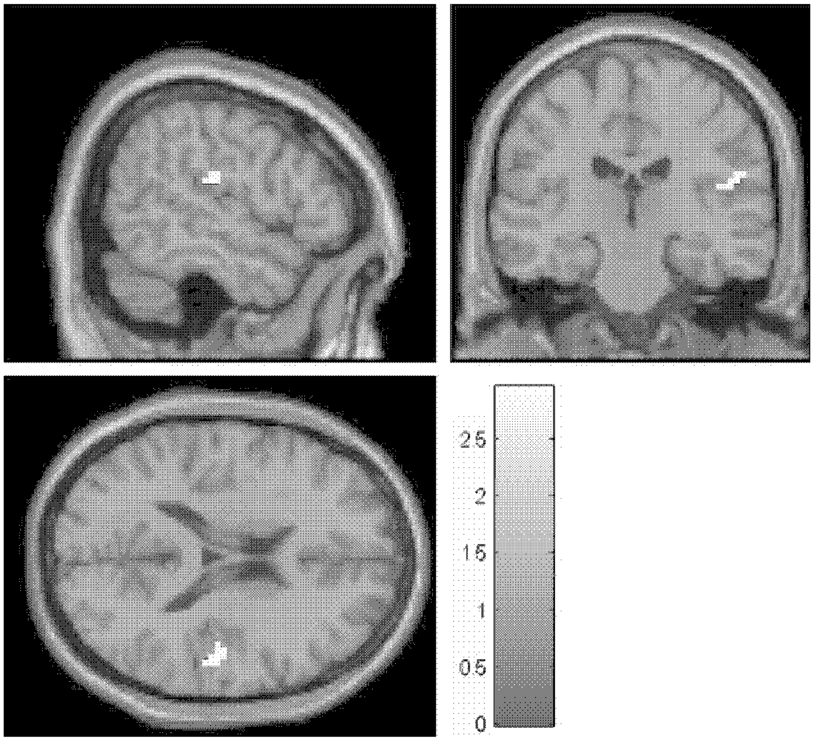

Figure 1 Brain areas with increased activation in the group control boys compared to control girls during the phonetic fluency.

Discussion

The current study aimed to investigate the neural substrate of VF in healthy adolescent boys and girls, and in adolescent MtFs and FtMs. Our results did not show significant differences in performance between healthy boys and girls, as both groups produced an equal number of words in the two conditions (phonetic and semantic fluency). To our knowledge this is the first study performed in early adolescence. In the literature, VF is generally described as a female-favoring task [19,20],

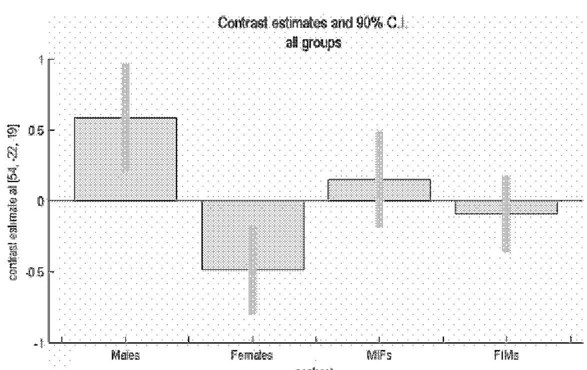

Figure 2 Peak-level activation of the four groups in the Right Rolandic Operculum

although it is conceivable that this sex difference only evolves later in adolescence, either under the influence of higher levels of or longer exposure to sex steroids. The young adolescents in the current study had been exposed to rising levels of sex steroids for only a relatively short period (their mean age was 14.6). As suggested by Sisk and Zehr [34] as well as Schulz [35], puberty may be considered an organizational period in itself with regard to brain development, and functional connectivity is likely to change during this period. To test this hypothesis, however, longitudinal designs are necessary with the aim of examining adolescents repeatedly until they reach adulthood.

Despite the fact that the control groups showed no sex differences in the amount of produced words, we did find sex differences in fMRI registered neuronal activity during the phonetic fluency condition. Control boys showed more activation in the right Rolandic operculum (BA 43) than girls. To our knowledge, activation in the right Rolandic operculum area has not been described previously to be involved in verbal fluency. The left Rolandic operculum is a small area caudally adjacent to Broca's area, and plays a role in speech production [36]. Tonkonogy and Goodglass [36] concluded from two case studies that small lesions in the left Rolandic operculum produce transient word finding or articulatory deficits. However, the current study shows differences in the right Rolandic operculum, and the explanation of the finding is not straightforward. Studies investigating verbal tasks have sought to explain sex differences with lateralization theory. This theory investigated by Witelson claims that men with clinical syndromes associated with prenatally low levels of testosterone show a higher prevalence of left-handedness and less functional asymmetry compared to other men [37]. For language-related tasks, men are thought to have a primarily left-centered unilateral representation of language, whereas women more often have a bilateral representation. These developmental changes of cerebral lateralization may result from prenatal exposure to testosterone (for an overview of the effects of testosterone on lateralization see meta-analysis from Pfannkuche et al. 2009 [38]). In the current study, however, we found more activation in boys in the right hemisphere, which therefore cannot be explained from the perspective of lateralization theory. Alternatively, one may argue that these lateralization differences may become fully established only during late adolescence and/or early adulthood, and that the observed sex difference in neuronal activation reflects increased effort or use of a different strategy in boys to achieve similar performance as girls. Of note, the Rolandic operculum has also been implicated in motor function, and case studies have shown that lesions in the Rolandic operculum cause a more "peripheral" deficit of speech articulation [39]. Koelsch et al. described the Rolandic operculum as a representation of the larynx and the pharynx [40]. In the current study, participants were specifically instructed not to talk or move their head or mouth during the MRI scan, but we cannot rule out the possibility that boys may have relied on subvocal rehearsal while generating words.

The second aim of this study was to investigate whether adolescents with GID would show neuronal activation patterns and VF performance in line with the experienced gender rather than their sex of birth. As hypothesized, VF performance data indeed showed that MtFs produced more words than control boys, but they also performed significantly better than the other two groups. This finding was not in line with our expectations. If brain development lies between both sexes as suggested by Cohen-Kettenis et al. [6], Gizewski et al. [13], and Rametti et al. [9], we would expect that MtFs performed worse on a female-favoring task than the girls. This was not the case, as their performance was superior to both control groups. No functional imaging study corroborates this finding, although one structural imaging study from Luders et al. [11] also showed a distinctive pattern of basal ganglia grey matter in a MtFs which does not fit the between-sexes hypothesis. Alternatively, we suggest that these VF performance differences may be explained, at least in part, by sample characteristics (e.g., a trendwise significant IQ difference between groups). Imaging data did not reveal significant differences between the adolescents with GID and controls in neuronal activation, although there was a trendwise significant linear increase within the Rolandic operculum between girls–FtM–MtF–boys. The finding that transsexuals or individuals with GID perform somewhere in-between the sexes is in itself not new: for instance, Cohen-Kettenis et al. showed in 1998 that MtFs performed better on a verbal memory test than male controls [6]. Furthermore, van Goozen et al. investigated visuospatial tests in transsexuals and found a significant linear performance increase from control females to FtMs to MtFs to control males. Also imaging studies such as those by Rametti et al. [9] and Zubiaurre-Elorza et al. [41] suggest that morphology of sex dimorphic brain structures in untreated individuals with GID lies in-between the two sexes. Rametti et al. showed in 2011 that microstructure patterns of white matter in untreated MtFs are intermediate between males and females. In addition, Zubiaurre-Elorza showed that MtFs have cortical thickness in sex differentiated brain areas that lies in-between the sexes, whereas the group of FtMs showed a volume size in the putamen that is more comparable with their desired sex than their natal sex. The current study extends these previous findings by demonstrating brain activity patterns in GIDs in between the control groups, even though these results only approached significance and hence need to be interpreted with caution.

It is not likely that brain functioning is entirely determined by prenatal exposure to hormones only. Activational effects of sex steroids may also play a role in these findings [42,43] and even nonsteroid related differences in development, such as genetic or environmental influence, such as differential treatment by parents or teachers, or exposure to strategies of class mates [44]. As a result, individuals with GID may not behave like peers of their birth sex, but also not entirely as peers of their experienced gender.

Because of some limitations our findings must be interpreted with some caution. Due to its

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cross-sectional design the current study lacks sensitivity to identify transient differences in development. As mentioned earlier, to investigate transient differences it would have been better to have used a longitudinal study design through adolescence. Also, sample sizes, in particular of the MtF group, are only modest. We attempted to include homogeneous groups of participants to reduce the risk of possible confounds, recruitment of which proved to be burdensome, however. Furthermore, some of our participants were left-handed, although distribution of handedness was similar across groups. Finally, we found a trendwise difference in the sample characteristics for IQ between the two groups. Although this difference is not significant, we should be aware that it might have had an effect on our findings.

This is the first brain imaging study to our knowledge investigating adolescents with GID. In addition, our group consists of hormone naïve participants, which is usually very difficult to accomplish when conducting research with adults. We believe that the homogeneously selected group of participants strengths the interpretation of the study. We have investigated a group of adolescents with GID with a typical early onset of transsexualism. Most studies so far did not report on the onset age of GID or investigated a heterogeneous group [11,14,27], which may have contributed to some of the inconsistent results.

The major implication of our findings is that with regard to verbal fluency, there are possibly transient sex differences in development during adolescence within a non-GID group. Adolescents with GID did not show similar effects. The trendwise linear increase in brain activity between the four groups may suggest differences in this area compared to their birth sex. Regarding regional brain activity, our findings are in line with the literature in adults with GID indicating structural and functional brain development which lies in-between the sexes. However, this hypothesis is in clear need of future replication in view of the VF performance data, with MtFs outperfoming the female control group, and also the male control group and FtMs. The next challenge will be to investigate this effect more in depth to understand more about the neuronal mechanisms of verbal abilities during the different stages of adolescence.

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References

1 American Psychiatric Association. Diagnostic and statistical manual of mental disorders (DSM–IV). 4th edition text rev. ed. Washington, DC: American Psychiatric Association; 2000.
2 WHO. The ICD–10 classification of mental and behavioral disorders: Clinical descriptions and diagnostic guidelines. Geneva: World Health Organization; 1992.
3 Zhou JN, Hofman MA, Gooren LJ, Swaab DF. A sex difference in the human brain and its relation to transsexuality. Nature 1995;378:68–70.
4 Kruijver FP, Fernandez-Guasti A, Fodor M, Kraan EM, Swaab DF. Sex differences in androgen receptors of the human

mamillary bodies are related to endocrine status rather than to sexual orientation or transsexuality. J Clin Endocrinol Metab 2001;86:818–27.
5 Garcia-Falgueras A, Swaab DF. A sex difference in the hypothalamic uncinate nucleus: Relationship to gender identity. Brain 2008;131:3132–46.
6 Cohen-Kettenis PT, van Goozen SH, Doorn CD, Gooren LJ. Cognitive ability and cerebral lateralisation in transsexuals. Psychoneuroendocrinology 1998;23:631–41.
7 van Goozen SH, Slabbekoorn D, Gooren LJ, Sanders G, Cohen-Kettenis PT. Organizing and activating effects of sex hormones in homosexual transsexuals. Behav Neurosci 2002;116:982–8.
8 Haraldsen IR, Opjordsmoen S, Egeland T, Finset A. Sex-sensitive cognitive performance in untreated patients with early onset gender identity disorder. Psychoneuroendocrinology 2003;28:906–15.
9 Rametti G, Carrillo B, Gomez-Gil E, Junque C, Zubiarre-Elorza L, Segovia S, Gomez A, Guillamon A. The microstructure of white matter in male to female transsexuals before cross-sex hormonal treatment. A DTI study. J Psychiatr Res 2011;45:949–54.
10 Yokota Y, Kawamura Y, Kameya Y. Callosal shapes at the midsagittal plane: MRI differences of normal males, normal females, and GID. Conf Proc IEEE Eng Med Biol Soc 2005;3:3055–8.
11 Luders E, Sanchez FJ, Gaser C, Toga AW, Narr KL, Hamilton LS, Vilain E. Regional gray matter variation in male-to-female transsexualism. Neuroimage 2009;46:904–7.
12 Berglund H, Lindstrom P, Dhejne-Helmy C, Savic I. Male-to-female transsexuals show sex-atypical hypothalamus activation when smelling odorous steroids. Cereb Cortex 2008;18:1900–8.
13 Gizewski ER, Krause E, Schlamann M, Happich F, Ladd ME, Forsting M, Senf W. Specific cerebral activation due to visual erotic stimuli in male-to-female transsexuals compared with male and female controls: An fMRI study. J Sex Med 2009;6:440–8.
14 Schoning S, Engelien A, Bauer C, Kugel H, Kersting A, Roestel C, Zwitserlood P, Pyka M, Dannlowski U, Lehmann W, Heindel W, Arolt V, Konrad C. Neuroimaging differences in spatial cognition between men and male-to-female transsexuals before and during hormone therapy. J Sex Med 2010;7:1858–67.
15 Savic I, Arver S. Sex dimorphism of the brain in male-to-female transsexuals. Cereb Cortex 2011;21:2525–33.
16 Maccoby EE, Jacklin CN. The psychology of sex differences. Palo Alto, CA: Stanford University Press; 1974.
17 Benton A. Multilingial aphasia examination. 2nd edition. Iowa City, IA: AJA Associates; 1983.
18 Lezak M. Neuropsychological assessment. 2nd edition. New York: Oxford University Press; 1983.
19 Bolla KI, Lindgren KN, Bonaccorsy C, Bleecker ML. Predictors of verbal fluency (FAS) in the healthy elderly. J Clin Psychol 1990;46:623–8.
20 Veroff AE. The neuropsychology of aging. Qualitative analysis of visual reproductions. Psychol Res 1980;41:2590–68.
21 Lanting S, Haugrud N, Crossley M. The effect of age and sex on clustering and switching during speeded verbal fluency tasks. J Int Neuropsychol Soc 2009;15:196–204.
22 Halari R, Sharma T, Hines M, Andrew C, Simmons A, Kumari V. Comparable fMRI activity with differential behavioural performance on mental rotation and overt verbal fluency tasks in healthy men and women. Exp Brain Res 2006;169:1–14.
23 Weiss EM, Siedentopf C, Hofer A, Deisenhammer EA, Hoptman MJ, Kremser C, Golaszewski S, Felber S, Fleischhacker WW, Delazer M. Brain activation pattern during a verbal fluency test in healthy male and female volunteers: A functional magnetic resonance imaging study. Neurosci Lett 2003;352:191–4.
24 Gauthier CT, Duyme M, Zanca M, Capron C. Sex and performance level effects on brain activation during a verbal fluency task: A functional magnetic resonance imaging study. Cortex 2009;45:164–76.
25 Nieder TO, Herff M, Cerwenka S, Preuss WF, Cohen-Kettenis PT, De Cuypere G, Haraldsen IR, Richter-Appelt H. Age of onset and sexual orientation in transsexual males and females. J Sex Med 2011;8:783–91.
26 Doorn CD, Poortinga J, Verschoor AM. Cross-gender identity in transvestites and male transsexuals. Arch Sex Behav 1994;23:185–201.
27 Flor-Henry P. EEG analysis of male to female transsexuals: Discriminant function and source analysis. Clin EEG Neurosci 2010;41:219–22.
28 Kreukels BP, Cohen-Kettenis PT. Puberty suppression in gender identity disorder: The Amsterdam experience. Nat Rev Endocrinol 2011;7:466–72.
29 APA. Diagnostic and statistical manual of mental disorders (DSM–IV). 4th edition text rev. ed. Washington, DC: American Psychiatric Association; 2000.
30 Tanner J. Growth and adolescence. Oxford, UK: Blackwell; 1962.
31 Wechsler D. Wechsler Intelligence scale for children. Third edition. San Antonio: The Psychological Corporation; 1991.
32 Wechsler D. Wechsler adult intelligence scale. Third edition. San Antonio: The psychological corporation; 1997.
33 Altena E, Van Der Werf YD, Sanz-Arigita EJ, Voorn TA, Rombouts SA, Kuijer JP, Van Someren EJ. Prefrontal hypoactivation and recovery in insomnia. Sleep 2008;31:1271–6.
34 Sisk CL, Zehr JL. Pubertal hormones organize the adolescent brain and behavior. Front Neuroendocrinol 2005;26:163–74.
35 Schulz KM, Molenda-Figueira HA, Sisk CL. Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. Horm Behav 2009;55:597–604.
36 Tonkonogy J, Goodglass H. Language function, foot of the third frontal gyrus, and rolandic operculum. Arch Neurol 1981;38:486–90.
37 Witelson SF. Neural sexual mosaicism: Sexual differentiation of the human temporo-parietal region for functional asymmetry. Psychoneuroendocrinology 1991;16:131–53.
38 Pfannkuche KA, Bouma A, Groothuis TG. Does testosterone affect lateralization of brain and behaviour? A meta-analysis in humans and other animal species. Philos Trans R Soc Lond B Biol Sci 2009;364:929–42.
39 Villa G, Caltagirone C. Speech suppression without aphasia after bilateral perisylvian softenings (bilateral rolandic operculum damage). Ital J Neurol Sci 1984;5:77–83.
40 Koelsch S, Fritz T, V Cramon DY, Muller K, Friederici AD. Investigating emotion with music: An fMRI study. Hum Brain Mapp 2006;27:239–50.
41 Zubiaurre-Elorza L, Junque C, Gomez-Gil E, Segovia S, Carrillo B, Rametti G, Guillamon A. Cortical thickness in untreated transsexuals. Cereb Cortex 2012 Aug 31. [Epub ahead of print] doi: 10.1093/cercor/bhs267.
42 O'Connor DB, Archer J, Hair WM, Wu FC. Activational effects of testosterone on cognitive function in men. Neuropsychologia 2001;39:1385–94.
43 Van Goozen SH, Cohen-Kettenis PT, Gooren LJ, Frijda NH, Van de Poll NE. Activating effects of androgens on cognitive performance: Causal evidence in a group of female-to-male transsexuals. Neuropsychologia 1994;32:1153–7.
44 Steensma TD, Kreukels BPC, de Vries ALC, Cohen-Kettenis PT. Gender identity development in adolescence. Horm Behav (under review).