Origins of gender differences in the human brain

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Chapter 2

Neural activation during mental rotation in complete androgen insensitivity syndrome: the influence of sex hormones and sex chromosomes

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ABSTRACT

Sex hormones, androgens in particular, are hypothesized to play a key role in the sexual differentiation of the human brain. However, possible direct effects of the sex chromosomes, that is, XX or XY, have not been well studied in humans. Individuals with complete androgen insensitivity syndrome (CAIS), who have a 46,XY karyotype but a female phenotype due to a complete androgen resistance, enable us to study the separate effects of gonadal hormones versus sex chromosomes on neural sex differences. Therefore, in the present study, we compared 46,XY men (n = 30) and 46,XX women (n = 29) to 46,XY individuals with CAIS (n = 21) on a mental rotation task using functional magnetic resonance imaging. Previously reported sex differences in neural activation during mental rotation were replicated in the control groups, with control men showing more activation in the inferior parietal lobe than control women. Individuals with CAIS showed a female-like neural activation pattern in the parietal lobe, indicating feminization of the brain in CAIS. Furthermore, this first neuroimaging study in individuals with CAIS provides evidence that sex differences in regional brain function during mental rotation are most likely not directly driven by genetic sex, but rather reflect gonadal hormone exposure.

INTRODUCTION

Sex differences have been described regarding human cognitive abilities (Maccoby and Jacklin 1974), regional brain structure (Ruigrok et al. 2014), and function (e.g., reviewed in Cahill 2006). However, the exact mechanisms involved in the sexual differentiation of the human brain and behavior are still being debated. The classic theory, for which the first evidence was provided by Phoenix et al. (Phoenix, Goy, Gerall and Young 1959), states that early androgen exposure during sensitive periods of neural development has organizing effects, masculinizing and defeminizing the brain, whereas the absence of androgens during these periods induces a female-typical neural development. Later studies in rodents suggested that estrogenic metabolites of androgens underlie many of these organizational effects (reviewed in Baum 1979), whereas evidence from studies in nonhuman primates (Wallen 2005), along with data from rare disorders in humans (Cohen-Bendahan et al. 2005; Baum 2006), has led to the hypothesis that, in humans, androgens act directly on the brain. From adolescence onwards, circulating levels of sex hormones are thought to have activational effects on brain and behavior. Apart from these hormonal effects, animal studies provide some evidence that sex chromosomes also have direct effects on certain sexually dimorphic behaviors (e.g. McCarthy and Arnold 2011), but this has not been well studied in humans yet.

A widely acknowledged cognitive sex difference has been demonstrated in spatial abilities, with the mental rotation task (MRT), a visuospatial task, showing the largest effect size (Linn and Petersen 1985; Voyer et al. 1995). This task reveals sex differences in performance to the advantage of men (Linn and Petersen 1985; Masters and Sanders 1993), and studies using functional magnetic resonance imaging (fMRI) revealed sexually differentiated neural activation patterns during the performance of this task, with generally higher levels of activation in parietal regions in men (Jordan et al. 2002; Weiss et al. 2003; Butler et al. 2006; Gizewski et al. 2006; Schöning et al. 2007; Hoppe et al. 2012). In addition, some studies have observed greater activation in frontal and temporal brain regions in women (Thomsen et al. 2000; Jordan et al. 2002; Weiss et al. 2003; Seurinck et al. 2004; Kucian et al. 2005; Butler et al. 2006; Gizewski et al. 2006; Schöning et al. 2007; Hoppe et al. 2012). Based on these findings from both behavioral and neuroimaging studies, different strategies for solving MRTs have been proposed: Women are thought to use a "serial", "analytic", and/or "top-down" approach, whereas men are thought to rely on a more automatic "bottom-up" and/or "gestalt" strategy (Thomsen et al. 2000; Jordan et al. 2002; Butler et al. 2006).

Most research on the factors involved in these sex differences in spatial abilities has focused on organizational and/or activational effects of sex hormones. Early "organizing" hormonal effects have been studied in individuals with atypical pre- and perinatal androgen levels. Women with congenital adrenal hyperplasia, who have an enzyme deficiency leading to excessive adrenal androgen production (which is treated shortly after birth), outperformed

their unaffected sisters on spatial tasks in childhood, adolescence, and adulthood (Puts et al. 2008; Berenbaum et al. 2012). Men who have had low androgen levels throughout life due to idiopathic hypogonadotropic hypogonadism showed impaired spatial abilities compared with control men (Hier and Crowley 1982). Effects of androgen replacement therapy in this group, however, are inconsistent (Hier and Crowley 1982; Zitzmann et al. 2001), which is probably due to small sample sizes. Similar studies in healthy subjects also provide evidence for organizing effects of prenatal hormones. Twin studies, inferring prenatal hormone levels based on intrauterine hormonal transfer between same-sex and opposite-sex twins, showed that females with a male co-twin performed better on an MRT than those with a same-sex twin (Vuoksimaa et al. 2010; Heil et al. 2011). Furthermore, higher levels of actual amniotic testosterone were associated with faster mental rotation performance in girls (Grimshaw et al. 1995). Taken together, these results suggest that spatial abilities are influenced by early gonadal hormone exposure. However, no conclusions can be drawn about whether these effects reflect the action of androgens directly or estrogens after aromatization of androgens.

Research into the possible "activational" effects of sex hormones on spatial ability has shown inconsistent results. Several studies investigating endogenous sex hormone levels (Moffat and Hampson 1996; Neave et al. 1999; Hooven et al. 2004; Kozaki and Yasukouchi 2008), natural fluctuations in these levels (e.g., during the menstrual cycle; Silverman and Phillips 1993; Phillips and Silverman 1997; Hausmann et al. 2000; Maki et al. 2002; Courvoisier et al. 2013), or the effect of exogenous sex hormone administration (Van Goozen et al. 1994, 1995; O'Connor et al. 2001; Aleman et al. 2004), have found associations between gonadal hormones and spatial abilities. Estrogens are thought to have a negative effect on the performance of spatial tasks, and most findings regarding testosterone suggest a positive effect in healthy women and a negative effect in healthy men, which can be explained by an inverted U-shaped curvilinear relationship (e.g. Aleman et al., 2004; Neave et al., 1999). However, not all studies have found these activational effects (Peters et al. 1995; Halari et al. 2005; Falter et al. 2006; Puts et al. 2010; Griksiene and Ruksenas 2011). The few studies that have investigated activational effects of sex hormones using fMRI have also rendered inconsistent results. Some studies have shown a positive association between hormone levels and mental rotation-related neural activity, but also negative or no associations have been reported (Dietrich et al. 2001; Schöning et al. 2007; Mendrek et al. 2011; Davis et al. 2013).

Another factor that differs between men and women, and therefore might be involved in the sexual differentiation of spatial abilities, is sex chromosomal composition. Whereas transgenic mouse models exist in which sex chromosome complement, that is, XX or XY, is unrelated to the gonadal sex of the animal, that is, ovaries or testes (e.g., Arnold and Chen 2009), similar studies in humans would be unethical to perform, since this would require experimental manipulation of gonadal hormone levels during development. Therefore,

studies in individuals with certain disorders of sex development (DSD), in which the development of chromosomal, gonadal, and/or anatomical sex is atypical (Hughes et al. 2006), can provide insights into the possible direct influence of sex chromosome genes unrelated to gonadal hormone exposure. Behavioral and neuroimaging studies on several aspects of cognition have been performed in individuals with Klinefelter Syndrome (KS), who have 1 Y and 2 or more X-chromosomes, and Turner Syndrome (TS), who lack (part of) 1 of the 2 X-chromosomes. Since in both disorders there is an aberrant number of sex chromosomes along with reduced androgen levels in KS and reduced estrogen levels in TS compared with healthy controls, it is difficult to establish whether results from these groups are attributable to effects of genes on the X or Y chromosome, chromosomal dosage, or sex hormone levels.

A DSD that involves a normal amount of sex chromosomes, and is therefore interesting to study, is the complete androgen insensitivity syndrome (CAIS). Individuals with CAIS have a 46,XY karyotype, but cannot respond to testosterone due to X-chromosomal genetic mutations resulting in a nonfunctional androgen receptor. This leads, despite normal to high male testosterone levels produced by their abdominal testes, to a female phenotype (Oakes et al. 2008). Studies using quantitative measures of psychosexual development indicate that individuals with CAIS are nearly always androphilic (sexually attracted to men), have a female gender identity, and show female-typical gender role behavior (Masica et al. 1971; Wisniewski et al. 2000; Hines et al. 2003). Spatial abilities in this group have only been studied by means of the Wechsler Intelligence Scale for Adults (WAIS) and for Children (WISC). On the spatial subtests of the WAIS, individuals with CAIS performed worse than control men and women (Imperato-McGinley et al. 1991), and Masica et al. (Masica, Money, Ehrhardt, and Lewis 1969) described the overall test score pattern of individuals with CAIS on the WAIS and WISC to be feminine, based on their superior performance on verbal compared with space-form ability-related subtests. So far, these results do not suggest any direct effects from sex chromosome genes, but they are in line with the hypothesis that in humans androgens, and not aromatized estrogens, play a role in the masculinization and/or defeminization of these behavioral and cognitive domains. However, taking into account the limited number of studies on this topic with generally small samples sizes, and the absence of results from cognitive tasks showing consistent sex differences, this issue needs further exploration.

Since, to date, no neuroimaging studies have been performed in individuals with CAIS, in the present study we compared brain activation during mental rotation in individuals with CAIS to control men and women matched for age and educational level. Comparing individuals with CAIS, who lack androgen action in the presence of XY-chromosomes, to 46,XY control men with typical male (high) androgen exposure and 46,XX control women with typical female (low) androgen exposure, provided us with the unique opportunity to study the separate effects of androgens and sex chromosome genes on the sexual differentiation of the brain. Furthermore, it enabled us to study whether possible masculinizing and/or defeminizing effects are caused by androgens directly, or by estrogens upon aromatization.

MATERIALS AND METHODS

Subjects

From an initial group of 85 subjects, 5 subjects (1 control man and 4 control women) were excluded because of excessive head movement during the fMRI session or anatomical abnormalities. The final subject sample consisted of 21 individuals with CAIS (46,XY; mean age 32.1; SD = 11.8), 30 control men (46,XY; mean age 31.7; SD = 9.5), and 29 control women (46,XX; mean age 31.2; SD = 9.4). The groups were matched for age and level of education. Participants reported no history of a serious medical, neurological, or psychiatric disease or MRI contraindications. All participants reported to have a right-hand preference for writing and, based on the Dutch Handedness Inventory (Van Strien 1992), all but one participant (ambidexter) had extreme right-handed scores. Control women did not use hormonal contraceptives, reported having a regular menstrual cycle, and were scheduled for participation in the period ranging from 4 days prior to, until 1 day after predicted ovulation, when estrogen levels are high.

The diagnosis CAIS was based on both clinical characteristics and mutation analysis of the androgen receptor gene using genomic DNA. In 7 of the 21 participants with CAIS, an unclassified variant of the androgen receptor gene mutation was found, and in 1 woman the analysis was still being performed at the time of writing. Since the clinical characteristics of these 8 individuals were compatible with CAIS, they were included in further analyses. Because the general medical advice is to remove the gonads before adulthood (Hughes et al. 2006), all individuals with CAIS included in the present study were gonadectomized and, with one exception, took hormone replacement therapy consisting of either estrogens (n = 16) or a combination of estrogens and progestins (n = 4), which is needed to compensate for the lack of gonadal sex hormone production.

Individuals with CAIS were recruited from the databases of the VU University Medical Center Amsterdam and the Erasmus University Medical Center-Sophia Children's Hospital Rotterdam, as well as from the support group DSDNederland. Flyers and advertisements in a local newspaper were used to recruit control subjects. The study was approved by the Medical Ethical Committee of the VU University Medical Center Amsterdam (application number NL32740.029.10), and all subjects gave their written informed consent according to the Declaration of Helsinki.

Gender-related psychological functioning questionnaires

Three questionnaires were used to obtain information about gender-related psychological functioning in all subjects. The Dutch version of the Klein Sexual Orientation Grid (Dessens et al. 1999; adapted from Klein et al. 1985) was used to assess sexual orientation, with a Likert scale ranging from 1 (exclusively homosexual) to 7 (exclusively heterosexual). For the assessment of gender identity (the sense of self as male or female) and gender role behavior

(participation in stereotypical masculine and feminine activities), a Gender Questionnaire (Callens et al. submitted; adapted from Hines et al. 2003) was used, with 6 questions added to the original questionnaire to cover all criteria for a gender identity disorder based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR; American Psychiatric Association, 2000). The scores range from 1 (sex-typical gender role behavior and gender identity) to 7 (sex-atypical gender role behavior and gender identity), with "sex-typical" referring to gender-of-rearing, which is male in control men and female in control women and individuals with CAIS. Scores for recalled childhood feminine and masculine toy and activity preferences were obtained from the Dutch version of the Activities Questionnaire (Callens et al. submitted; adapted from Hines et al. 2003), with higher scores indicating a greater preference (sum score range 4-20).

Hormone assessment

To assess levels of circulating hormones in all participants and to determine the actual menstrual cycle phase of the control women at the day of testing, venous blood samples were obtained within a maximum of 2 h prior to the fMRI experiment. The blood samples were centrifuged and serum was stored at -20 °C. After the samples of all participants had been collected, analyses took place at the Endocrine Laboratory of the VU University Medical Center Amsterdam. Total testosterone (TT) levels were measured using ID-LC-MS/MS, as described earlier (Bui et al. 2013). A fluorescent immunoassay (Delfia, Perkin Elmer, Finland) was used to measure serum levels of estradiol. Progesterone, luteinizing hormone (LH), and sex hormone-binding globuline (SHBG) were measured using an automated immunoassay (Architect, Abbott, IL, USA). The levels of free testosterone (FT) were calculated with the formula described by Vermeulen et al. (Vermeulen, Verdonck and Kaufman 1999), using the serum levels of TT, SHBG and a standard albumin concentration of 43 g/L.

Experimental procedure

All participants received instructions for the MRT at the day of testing and, to ensure the instructions were understood, performed a practice trial before the MRI session was started. These instructions were repeated in the scanner, immediately preceding the fMRI experiment. Instructions and test materials were presented using E-prime (Psychology Software Tools, Pittsburgh, PA, USA) and projected on a screen, visible through a mirror attached to the head coil. Participants responded to the stimuli using MRI-compatible button boxes.

The stimuli used in this experiment were colored 3D-objects (Shepard and Metzler 1971) with varying degrees of rotation ranging from 45º to 315º. The stimuli were presented in an alternating block-design with 5 rotation or control trials in each block. Each trial consisted of a fixation screen (a white fixation cross in the center of a black background) displayed for 1 s, immediately followed by a control or rotation stimulus. Rotation stimuli consisted of 2 individually rotated 3D objects that were either identical or mirrored versions of each other.

Participants were asked to respond to identical objects by pressing the right button box and to mirrored objects by pressing the left button box. The control stimuli, controlling for the visual properties of the stimuli and motor responses, consisted of one 3D-object with an arrow underneath it, pointing either left or right, and participants were instructed to press the button corresponding to the direction the arrow was pointing at. Response latency (reaction time, RT) and accuracy were recorded. The next trial started after a response or, in case of no response, after 20 s. The total task duration was 300 s.

Image data acquisition

Image data were acquired with a 3.0-T GE Signa HDxt scanner (General Electric, Milwaukee, WI, USA). After high order shimming of the magnetic field, fMRI data were acquired using an echo-planar imaging (EPI) sequence (repetition time [TR] 2100 ms, echo time [TE] 30 ms, flip angle [FA] 80°, 64x64 matrix, field of view [FoV] 240 mm, 40 slices, 3.75x3.75x3.0 mm voxel size). A high-resolution T1-weighted anatomical image was acquired using a 3D fast spoiled gradient echo (FSPGR) sequence (TR 7.8 ms, TE 3.0 ms, FoV 250 mm, and slice thickness 1 mm). Foam padding was used in the head coil to reduce head motion.

Data analysis

Sample characteristics and behavioral data

Statistical analysis of the sample characteristics (age, level of education, gender-related psychological functioning and serum hormone levels) and behavioral data was performed with IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). Scores for the level of education ranged from 1 (primary school) to 8 (university degree), and both the current level of education at the day of testing and the expected level of education once the participant had finished the current educational trajectory (if applicable), were taken into account. Mean RT and accuracy (percentage correct responses) on rotation and control trials were calculated for each participant. Omitted trials were discarded from the behavioral data analyses. One male participant had to be excluded from the analysis of accuracy data, due to a malfunctioning button box.

Kruskal-Wallis and post hoc Mann-Whitney *U*-tests were used for the between-group analyses, because the assumptions for the use of parametric tests were violated in all of these variables. For all data, results were considered significant at a $P < 0.05$ threshold. Bonferroni correction for multiple comparisons was applied to all reported post hoc test statistics.

fMRI data

Preprocessing and statistical analysis of the fMRI data were performed using Statistical Parametric Mapping 8 (SPM8; Wellcome Trust Center for Neuroimaging, Institute of Neurology at

UCL, UK), and a software suite of MATLAB (version R2011a; MathWorks, Inc., Natick, MA, USA). First, the origin of the anatomical and those of the EPI images were manually reoriented to roughly match the AC-PC line. Next, several preprocessing steps were performed in the following order: EPI images were slice-time corrected to the middle slice and realigned to the first image to correct for slight head movements; the anatomical image was co-registered to the mean EPI image; the EPI images were normalized to the MNI template using normalization parameters derived from the segmented anatomical image; a Gaussian kernel (full-width at half maximum = 8 mm) was used to smooth the EPI images.

At the within-subject level, a boxcar function convolved with the canonical hemodynamic response function was used to model the blood oxygen level-dependent (BOLD) response during rotation and control blocks. To account for motion-related artifacts, "scan nulling" regressors for each between-volume head movement exceeding 0.5 mm, and 6 motion regressors, obtained during realignment, were added to this model. Individual contrast images for condition-specific effects were defined by subtracting the control condition from the rotation condition, and were entered into second-level random-effects analyses.

An analysis of covariance (ANCOVA), with individual mean RT to rotation stimuli as a covariate of no interest, was used to analyze the within-group main effect of the task (rotation > control) and between-group effects. To focus only on those brain areas that are involved in mental rotation, the results from the whole-brain between-group analyses were masked with the across-groups main effect of the task ($P < 0.05$ FWE-corrected). Furthermore, 2 regions of interest (ROIs), based on a meta-analysis of neuroimaging studies on mental rotation (Zacks 2008), were selected. The Nielsen and Hansen's volume of interest BrainMap database (Nielsen and Hansen 2002) was used to select the areas in and immediately adjacent to the bilateral intraparietal sulcus. With the MarsBar Toolbox (Brett et al. 2002), these anatomical ROIs were masked with the across-groups main effect of the task ($P < 0.05$ FWE-corrected), resulting in a left and right parietal ROI. A similar technique for ROI definition to investigate sex differences on an MRT was previously described by Hoppe et al. (Hoppe et al. 2012). Finally, correlations between mental rotation-related brain activation and serum levels of estradiol and FT were investigated using within-group linear regression analyses. For individuals with CAIS the correlation with FT was not analyzed, because of the known insensitivity to testosterone. Whole-brain results were again masked with the main effect of the task and ROI analyses were performed with the above-described parietal ROIs. Results were considered significant using a statistical threshold of $P < 0.05$ (FWE-corrected) for the whole-brain and ROI analyses.

RESULTS

Sample characteristics and behavioral data

Sample characteristics and behavioral data are summarized in Table 1. The 3 groups did not differ on age and level of education. All groups showed high, that is, heterosexual, scores on sexual orientation (currently and during puberty), with a more "extreme" current heterosexual orientation in control men than in individuals with CAIS ($P = 0.009$), and control women (trend: $P = 0.090$). On measures of current and lifetime gender identity and gender role behavior, scores for control men were in ranges comparable to reference groups of healthy men, and for control women and individuals with CAIS to reference groups of healthy women. Control men showed a greater childhood preference for masculine toys and activities than control women ($P < 0.001$) and individuals with CAIS ($P = 0.003$), and the latter 2 groups showed a greater preference for feminine toys and activities than control men (both $P < 0.001$).

There was a significant effect of group on RT during rotation trials, with men responding faster than individuals with CAIS ($P = 0.021$). There was no main effect of group on accuracy scores for rotation trials, or for accuracy and RT during the control trials.

Serum hormone levels

As expected, a significant effect of group was found for serum levels of estradiol, TT, and FT (Table 1). For TT and FT, all 3 between-group tests were significant, showing that men had higher levels than control women and individuals with CAIS, with the latter group displaying the lowest levels (all P-values <0.001). Estradiol levels in men were significantly lower than in control women and individuals with CAIS ($P < 0.001$), the latter 2 groups did not differ significantly. Group differences in serum levels of progesterone and LH could not be investigated, because an exact value was missing due to serum hormone levels below the limit of quantitation (2 nmol/L or U/L) for multiple participants.

Based on serum levels of LH, estradiol and progesterone, 9 control women were in the mid-cycle/ovulatory phase (LH >14 U/L and/or estradiol >620 pmol/L), 3 in the luteal phase (progesterone >4 nmol/L), 1 in between the ovulatory and luteal phase (LH >14 U/L and progesterone >4 nmol/L), and 16 in the follicular phase of the menstrual cycle (progesterone <3 nmol/L).

Table 1 Sample characteristics, MRT performance and serum hormone levels

	Control men (n = 30)	Control women (n = 29)	CAIS (n = 21)	χ^2	P-value
Sample characteristics, mean (SD)					
Age	31.7 (9.5)	31.2 (9.4)	32.1 (11.8)	0.006	0.997
Level of education (C)	5.5 (1.8)	6.1 (1.5)	5.9 (1.6)	1.867	0.393
Level of education (E)	6.3 (1.9)	6.5 (1.7)	6.1 (1.6)	0.627	0.731
Sexual orientation (C)	6.92 (0.19)	6.81 (0.25)	6.51 (0.63)	9.444	**0.009**
Sexual orientation (P)	6.86 (0.30)	6.84 (0.27)	6.60 (0.63)	2.144	0.342
Gender identity (C)	1.04 (0.10)	1.11 (0.18)	1.09 (0.21)	4.079	0.130
Gender identity (L)	1.07 (0.14)	1.12 (0.20)	1.12 (0.31)	1.859	0.395
Gender role (C)	1.83 (0.79)	1.93 (1.10)	1.38 (0.67)	4.963	0.084
Gender role (L)	1.90 (0.80)	2.03 (1.05)	1.43 (0.68)	5.912	0.052
Masculine toys/activities	16.00 (2.12)	12.69 (2.55)	12.71 (3.49)	23.090	**< 0.001**
Feminine toys/activities	6.53 (1.62)	12.51 (2.97)	13.33 (3.24)	48.421	**< 0.001**
MRT performance					
Reaction time (s)[a]	4.61 (1.99)	5.34 (2.44)	6.55 (2.43)	8.132	**0.017**
Accuracy[a,b]	75.1 (21.9)[c]	76.1 (12.7)	76.0 (19.7)	1.119	0.571
Serum hormone levels					
Estradiol (pmol/L)	82.28 (18.20)	387.60 (312.63)	276.15 (210.93)	36.972	**< 0.001**
Total testosterone (nmol/L)	13.26 (5.96)	0.98 (0.33)	0.34 (0.15)	68.745	**< 0.001**
Free testosterone (pmol/L)	297.60 (128.40)	15.20 (5.67)	3.64 (1.73)	69.285	**< 0.001**

Note: Mean and standard deviation (SD) for serum levels of progesterone and LH could not be calculated, because multiple participants had serum levels below the limit of quantitation of 2 nmol/L or U/L, and therefore an exact value was missing. Bold P-values represent a significant (P < 0.05) between group result.
C: current; E: expected; P: puberty; L: lifetime.
[a]With respect to rotation stimuli
[b] % correct responses
[c] for accuracy n = 29

Neuroimaging data

Main effect rotation versus control condition

An overall similar activation pattern during mental rotation after subtraction of activation during the control condition was found in the 3 groups (Fig. 1). All groups showed bilateral activations in the parietal lobe, predominantly in inferior and superior regions, extending into the occipital lobe. Activations were also observed in frontal areas, to a large extent in the precentral, and superior and middle frontal gyrus.

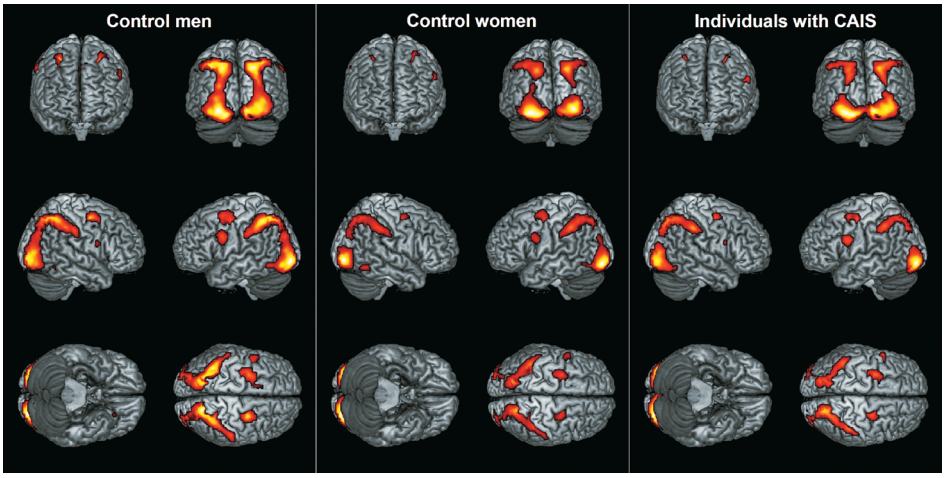

Figure 1 Significant activations during the rotation condition after subtraction of the control condition. Thresholded activation maps ($P < 0.05$ FWE-corrected) are displayed on a standard anatomical template (ch2bet) using MRIcron (Rorden et al. 2007).

Between-group activation differences

Between-group ROI analyses revealed sex differences between control men and women (Table 2 and Fig. 2). Control men showed significantly more activation than control women in the left inferior parietal lobe, and a trend was observed in the right inferior parietal lobe. No areas of greater activation were observed in the reversed contrast.

When including individuals with CAIS in the between-group comparisons on the 2 ROIs (Table 2), individuals with CAIS showed a similar activation as control women when compared with control men, who again showed more activation in the left inferior parietal lobe. Figure 2 presents the between-group differences and their overlap in the inferior parietal lobe. Other between-group comparisons in the 2 ROIs revealed no significant results. None of the whole-brain between-group comparisons, using the main effect of the task across groups to mask the results, yielded a significant result using a $P < 0.05$ FWE-corrected threshold.

Table 2 Differential mental rotation-related brain activations between groups

Group	Brain region	Hemisphere	Cluster size	MNI-coordinates x	y	z	$P_{FWE\text{-}corrected}$	T-value
M > W	Inferior parietal lobe	L	486	-36	-49	49	**0.035**	3.72
				-27	-52	52	0.052	3.59
	Inferior parietal lobe	R	243	33	-52	52	0.094	3.40
W > M	—	—	—	—	—	—	—	—
M > CAIS	Inferior parietal lobe	L	297	-33	-49	49	**0.047**	3.62
CAIS > M	—	—	—	—	—	—	—	—
W > CAIS	—	—	—	—	—	—	—	—
CAIS > W	—	—	—	—	—	—	—	—

Note: Cluster sizes in mm^3 based on results using an uncorrected whole-brain threshold of P = 0.001. Results from analysis within the bilateral parietal ROIs. Bold P-values represent a significant (P < 0.05 FWE-corrected) between group result.
M: control men; W: control women; CAIS: individuals with CAIS.

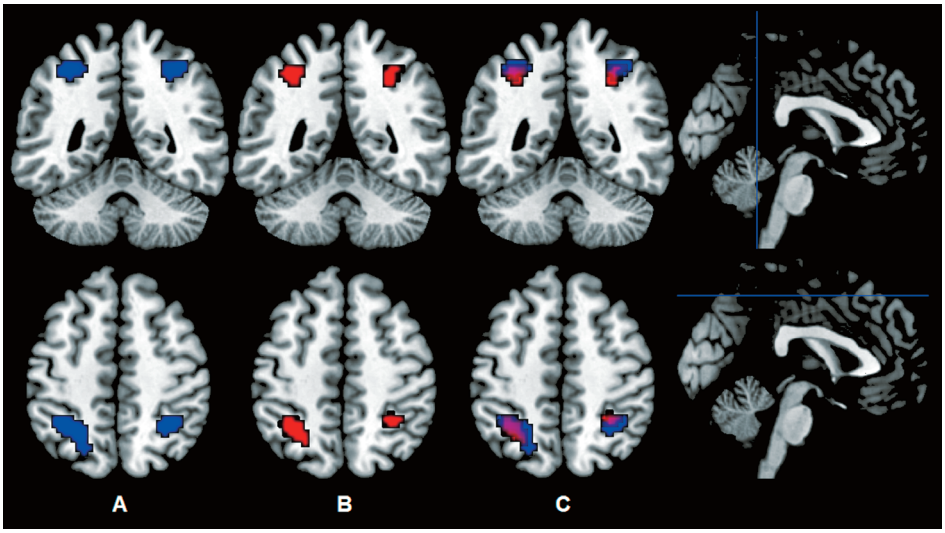

Figure 2 Between-group differences in activation during the rotation condition after subtraction of the control condition. Blue = control men > control women; left and right inferior parietal lobes (A). Red = control men > individuals with CAIS; left inferior parietal lobe (B). The overlap of the between-group differences in activation is demonstrated in C. For illustrative purposes, whole-brain results, masked with the bilateral parietal ROIs, are displayed with an uncorrected threshold of P < 0.005 (k = 20) on a standard anatomical template (ch2better) using MRIcron (Rorden et al. 2007).

Correlations with estradiol and FT

None of the within-group linear regression analyses with serum levels of estradiol and calculated FT revealed significant correlations, positive or negative, using the P < 0.05 FWE-corrected threshold in both the whole-brain and ROI analyses. For exploration, we also

lowered the threshold to $P < 0.001$ uncorrected with an extend threshold of $k = 10$ adjacent voxels for the whole-brain regression analyses, but again no voxels exceeded this threshold.

DISCUSSION

In the present study, we included a unique group, that is, individuals with CAIS, to investigate the role of gonadal hormones and sex chromosome genes on the sexual differentiation of regional brain function during an MRT. Our findings suggest that the reported sex difference in brain functioning during mental rotation is not directly driven by genetic sex, but may be attributable to gonadal hormone exposure, most likely androgens.

The overall pattern of brain activation during mental rotation, comprising bilateral activation in parietal, frontal, and occipital regions in each group, is consistent with results from a meta-analysis on neuroimaging studies during mental rotation (Zacks 2008). As expected, a sex difference was found between the control groups; control men showed a greater activation in the left inferior parietal lobe than control women, and a similar, though not significant, effect was observed in the right hemisphere. This finding is in line with previous reports of greater neural activation in the left (Weiss et al. 2003; Gizewski et al. 2006; Schöning et al. 2007; Hoppe et al. 2012) and in the right (Gizewski et al. 2006) inferior parietal lobes in men than in women. We found no areas of greater activation in control women than in control men.

To investigate whether sex chromosomes are involved in these sexually differentiated neural activations during mental rotation, we compared individuals with CAIS to the control groups. No differences were found between control women and individuals with CAIS. In the comparison with control men, however, we found that men showed greater activation in the left inferior parietal lobe, whereas the reversed contrast did not reveal areas showing greater activation. Since these results are similar to those for the comparison of control men and women, we may conclude that individuals with CAIS show a female-typical parietal neural activation, which indicates that not only the body, but also the brain is, at least partially, feminized in CAIS. In combination with the absence of any male-typical neural features in individuals with CAIS, these results argue against a direct role for sex chromosome genes. The reported neural sex differences in the present study are, therefore, more likely to result from different levels of, or neural responsiveness to, gonadal hormones.

Because individuals with CAIS produce testosterone, which is converted to estrogens through aromatization, and have normally functioning estrogen receptors, possible masculinizing and/or defeminizing effects of estrogens (as is seen in several rodent species) should be observable. However, the absence of male-typical results in this group supports the hypothesis that in humans androgens, and not estrogens, are the masculinizing and/or defeminizing hormones. Based on this study, it is not possible to distinguish between or-

ganizational and activational effects of these androgens, as the insensitivity to androgens is already present prenatally and continues throughout life. Furthermore, individuals with CAIS have higher serum estrogen levels than men due to aromatization of testosterone when the gonads are still in situ (Hughes and Deeb 2006), and due to estrogen replacement therapy after gonadectomy. Since all individuals with CAIS in this study were gonadectomized and, with one exception, using estrogens, a feminine pattern of neural activation in individuals with CAIS could also be attributed to estradiol. This would be in line with studies that have proposed a role for estrogens in mental rotation-related neural activation and performance (e.g., Maki et al. 2002; Schöning et al. 2007; Kozaki and Yasukouchi 2008); however, other studies have not found these activational effects (e.g., Peters et al. 1995; Halari et al. 2005; Griksiene and Ruksenas 2011).

A methodological limitation of the present study is that, due to the significant between-group differences in serum estradiol and TT and FT levels, hormone levels could not be included as covariates in the between-group analyses, nor could they be used in a regression analysis including subjects from all 3 groups. We were able to perform regression analyses in each group separately, which did not reveal any correlations between neural activation during mental rotation and serum levels of estradiol and FT, suggesting that within-group variations in brain response were most likely not driven by activational effects of current sex hormone levels. However, since the between-group differences in these hormone levels were much larger than the within-group variation, we cannot rule out activational effects based on these results. Therefore, although no evidence was found for any direct genetic effects and our results thus point to the involvement of hormonal factors, we can only speculate about the exact role of sex hormones in the reported differences in neural activity between the groups.

In this respect, it would be interesting to study gonadectomized individuals with CAIS who are not receiving estrogen replacement therapy. This would enable us to investigate whether the female-typical neural activation pattern in individuals with CAIS is actually caused by the lack of androgen action, or whether activational estrogenic factors are involved. However, adult individuals with CAIS who have undergone gonadectomy need estrogen replacement to maintain their health, and specifically to prevent osteoporosis. It would thus be unethical to ask them to stop hormone replacement for a period of time for research purposes only.

An alternative explanation of our between-group results that needs to be considered is based on socialization effects on spatial abilities. Exposure to typically masculine toys and activities is thought to have enhancing effects on spatial task performance (e.g. Connor and Serbin 1977; Baenninger and Newcombe 1989). Since recalled childhood toy and activity preferences were sex-typical in the subject groups, that is, there was a greater preference for masculine toys and activities in control men, and for feminine toys and activities in control women and individuals with CAIS, a potential socialization effect cannot be ruled out. Gen-

der stereotypes about male superiority on spatial tasks have also been proposed to affect the behavioral sex difference on the MRT (Hausmann et al. 2009). To minimize potential gender stereotype effects on the sex differences in neural activation, the participants in the present study were not informed about the sex difference on the MRT before participation.

Unlike previous studies, we did not find a significant difference in performance between control men and women. Control men performed significantly faster than individuals with CAIS, but not when compared with control women. Jordan et al. (Jordan et al. 2002) have shown that behavioral sex differences are not a requirement for the detection of neural sex differences during mental rotation, indicating that different neural strategies may underlie similar behavioral outcomes. Furthermore, even though we did not replicate the behavioral sex differences between the control groups, a similar computerized MRT has been proved to be a valid measure of mental rotation (Voyer et al. 2006).

We have tried to carefully select an adequate type of control stimulus, taking into account the need for similar visual properties as the rotation stimuli and the requirement of a motor response, in order to capture only rotation-related activation. A limitation of the control stimuli selected for the present study is that subjects could have only focused on the arrow below it and not so much on the 3D-object, which means that the control stimuli might not have sufficiently replicated the visual properties of the rotation stimuli. The results from the main effect of the task, however, showed a similar overall pattern of activation in all 3 groups in line with the results from previous studies on mental rotation (Zacks 2008).

Taken together, this first fMRI study in individuals with CAIS indicates that the sexual differentiation of visuospatial neural activation is not directly influenced by sex chromosomal composition, but is likely to be determined by gonadal hormone exposure, presumably by androgens. This conclusion is based on the female-like activation pattern in mental rotation-related brain areas in individuals with CAIS, which also demonstrates that CAIS not only leads to feminization of the body, but also of (parts of) the brain. Since this is the first neuroimaging study comparing individuals with CAIS to control men and women to investigate the factors involved in neural sexual differentiation, future studies using this unique model are likely to provide further understanding of the role of sex chromosome genes and gonadal hormones in other sexually differentiated cognitive functions and regional brain morphology.

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