

Brain responses to sexual images in 46,XY women with complete androgen insensitivity syndrome are female-typical



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ABSTRACT

Androgens, estrogens, and sex chromosomes are the major influences guiding sex differences in brain development, yet their relative roles and importance remain unclear. Individuals with complete androgen insensitivity syndrome (CAIS) offer a unique opportunity to address these issues. Although women with CAIS have a Y chromosome, testes, and produce male-typical levels of androgens, they lack functional androgen receptors preventing responding to their androgens. Thus, they develop a female physical phenotype, are reared as girls, and develop into women. Because sexually differentiated brain development in primates is determined primarily by androgens, but may be affected by sex chromosome complement, it is currently unknown whether brain structure and function in women with CAIS is more like that of women or men. In the first functional neuroimaging study of (46,XY) women with CAIS, typical (46,XX) women, and typical (46,XY) men, we found that men showed greater amygdala activation to sexual images than did either typical women or women with CAIS. Typical women and women with CAIS had highly similar patterns of brain activation, indicating that a Y chromosome is insufficient for male-typical human brain responses. Because women with CAIS produce male-typical or elevated levels of testosterone which is aromatized to estradiol these results rule out aromatization of testosterone to estradiol as a determinate of sex differences in patterns of brain activation to sexual images. We cannot, however, rule out an effect of social experience on the brain responses of women with CAIS as all were raised as girls.

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There is considerable interest in understanding the origins of human psychological sex differences. Gender-related socialization clearly plays a role, and there is good evidence that sex hormones, especially prenatal androgens, also influence sex-related behavior (Berenbaum and Beltz, 2011; Hines, 2011; Ruble et al., 1998). In many species estradiol produced by the aromatization of testosterone has been shown to be important for masculinization of behavior, but there is little evidence to support a role for estradiol in human sexual differentiation (Wallen and Baum, 2002). Similarly, there is little evidence for a role of sex chromosomes in the sexual differentiation of human behavior, although they have been suggested to play an important role in sexual differentiation of behavior in a variety of species (Arnold, 2004, 2009).

A unique opportunity to assess the role of the Y chromosome versus androgens, estrogens, or socialization in human sexually differentiated behavior and brain responses is provided by complete androgen insensitivity syndrome (CAIS) (Arnold, 2004; Berenbaum et al., 2009;

McCarthy and Arnold, 2011; Quigley et al., 1995; Wisniewski et al., 2000). Individuals with CAIS have a 46,XY karyotype, testes that secrete male-typical or exaggerated amounts of testosterone prenatally and post-natally, elevated estrogen levels derived from androgens, but lack functional androgen receptors due to mutations of the androgen receptor (AR) gene (Cheikhlard et al., 2009; Hughes et al., 2012; Quigley et al., 1995).

They are thus born with female external genitalia, develop a female phenotype, are reared as girls, and undergo a feminizing puberty as a result of the aromatization of their testosterone to estradiol (Cheikhlard et al., 2009). Behavior in women with CAIS appears to be female-typical (Hines et al., 2003; Wisniewski et al., 2000), as do their spontaneous otoacoustic emissions, a sexually differentiated aspect of the auditory system thought to reflect sex differences in brain stem organization (Wisniewski et al., 2014). There have been, however, no studies of brain structure or patterns of neural activation in women with CAIS. Evidence of male-typical brain responses in women with CAIS relative to those of control women could reflect altered expression of genes on the sex chromosomes due to presence of a Y chromosome (Arnold, 2009), absence of a second X chromosome, actions of androgens not mediated by classic nuclear receptor pathways, or a role for estradiol

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derived from androgens in human masculinization (Balthazard and Ball, 2012; Wallen and Baum, 2002). By contrast, female-typical responses could reflect absence of organizational or activational androgen effects, feminizing effects of estrogens, or result from female-typical socialization characteristic of women with CAIS (Hines, 2011).

To address this question, we examined behavioral and brain responses to sexually arousing stimuli, given the prominent sex differences in this domain (Gizewski et al., 2009; Hamann et al., 2004; Ponseti et al., 2006; Sylva et al., 2013; Whittle et al., 2011). In the first functional neuroimaging study of women with CAIS, we used fMRI to examine brain responses to sexually arousing images compared to those typical of control men and women. Given female-typical behavioral profiles in women with CAIS (Hines et al., 2003), we hypothesized that these women would exhibit female-characteristic fMRI patterns of responses to sexual images, consistent with neural sex differences reflecting exposure and response to androgens rather than X or Y chromosome dosage (Arnold, 2009; Cahill, 2006; Lentini et al., 2012).

We examined this hypothesis using two converging approaches. First, we examined fMRI responses to images of heterosexual sexual behavior, self-reported as comparably sexually arousing by women with CAIS and control men and women. We previously reported that men exhibit substantially greater amygdala activation than do women to such stimuli, despite similar subjective arousal ratings (Hamann et al., 2004). The second approach focused on differential responses to same vs. other-sex images in each group. Heterosexual and homosexual men exhibit substantially greater brain activation to their preferred sexual stimuli vs. nonpreferred or neutral images and a striking absence of greater regional activation to nonpreferred vs. preferred sexual stimuli, a phenomenon termed “category-specificity” (Chivers, 2005; Chivers et al., 2004; Ponseti et al., 2006; Safron et al., 2007; Sylva et al., 2013). Accordingly, we contrasted brain activation in response to photographs of nude women and nude men between control heterosexual men and women to establish heterosexual male and female category-specific brain activation profiles. We then used these characteristic response profiles to determine whether women with CAIS exhibit brain responses more similar to those of heterosexual women or heterosexual men. Finally, because physiological sexual arousal responses are substantially less category-specific in heterosexual women than in heterosexual men (Chivers, 2005; Chivers and Bailey, 2005; Chivers et al., 2004; Rupp and Wallen, 2008), we predicted that category-specific brain responses in control women and women with CAIS would be significantly weaker and more limited in anatomical extent than those of control men.

Materials and methods

Subjects

We studied 13 women (mean age 28.2 years [SD = 4.8]), 13 men (mean age 26.5 years [SD = 6.7]) and 13 women with CAIS (mean age 37.7 years [SD = 10.8]). The study was approved by the Institutional Review Boards of the participating institutions, and participants provided informed consent. Comparison (control) subjects were recruited from the local community via advertisements. Women with CAIS were recruited from a support group (AISDSD.org) and were identified by self-report of their medical diagnoses. Members of AIS-DSD are typically well educated about the specifics of their diagnosis, based on detailed research into their personal medical histories, including karyotype, hormonal profile, and clinical features. There is a good correlation between androgen receptor genotype and phenotype in CAIS (Cheikhelard et al., 2009). Subjects were prescreened with a sexual desire and behavior questionnaire to determine that they were heterosexual (opposite-sex sexual desire and sexual experiences). We specifically chose to compare groups of self-identified heterosexual individuals to minimize confounding of the comparisons by sexual orientation.

fMRI scanning

Scanning was conducted using a 3 T Siemens Tim Trio MRI system using a 12-channel parallel head coil. Functional data were acquired using a gradient-echo, echo-planar pulse sequence (TR = 2000 ms, TE = 30 ms, 37 horizontal interleaved slices, 3 × 3 × 3 mm voxel size). Each run contained 182 scans (total run duration 364 s); 3 additional scans collected prior to the experimental task were discarded to allow for magnetic field stabilization. High-resolution (1 × 1 × 1 mm) T1-weighted (MP-RAGE) structural images were collected for anatomical visualization.

Stimuli and tasks

During scanning, subjects viewed six types of photographic stimuli: (1) heterosexual couples engaged in sexual activity, (2) female nudes, (3) male nudes, (4) female couples engaged in sexual activity, (5) male couples engaged in sexual activity, and (6) a control, nonsexual condition that showed pleasant social interaction between partially or fully clothed men and women with no overt sexual content (neutral stimulus condition). Female and male nude stimuli did not depict sexual activity. The sexual activity stimuli depicted heterosexual sexual activities that were selected to be equally arousing to women and men, and these were drawn from a previous study (Hamann et al., 2004), supplemented by additional stimuli from professional sources. Approximately 1/3 of the male nude stimuli displayed the male's penis and with a few exceptions the penis was erect. All of the female nudes had intact pubic hair that masked the details of their genitals. Thus the nude male and female stimuli were not equated for prepotency and may differ from those used in studies from other laboratories (Chivers et al., 2007; Spape et al., 2014). However, these stimulus differences are likely not important for our current purposes of assessing group differences. Stimuli were presented via an LCD side-projection system and mirror. Button-press responses were made using a fiber-optic pad.

Stimuli were presented in two runs, in pseudorandom order in an event-related fMRI design with jittered inter-trial intervals. In each run, 72 stimuli were presented (12 from each stimulus category). Each trial began with presentation of a picture stimulus for 1.5 s, followed by a response screen (like | neutral | dislike) for 1.5 s, and finally a variable inter-trial fixation interval (a white “+” on a black background) for either 1.5 or 2.5 s (5 s. mean inter-trial interval). Subjects were instructed to view each picture, to experience any thoughts or feelings it might elicit, and to press buttons indicating whether they liked, disliked, or felt neutral towards the picture contents. The order of buttons corresponding to these ratings was counterbalanced across subjects. Subjects maintained visual fixation during the variable inter-trial interval.

After scanning, subjects completed sexual arousal ratings for all stimuli that had been viewed during scanning, outside the scanner on a computer, alone with no experimenter present. Twenty-four photographic stimuli from each of the six stimulus categories were rated. Each stimulus was shown full-screen for an unlimited duration while subjects rated their sexual arousal to it on a 1–5 scale (1: no sexual arousal; 5 highest) in response to the question, “How sexually arousing is the picture to you?”. Post-scan arousal ratings from a single outlying control woman who rated every stimulus as “1” were excluded from the behavioral analysis.

Behavioral and imaging analyses

Behavioral ratings were analyzed using mixed-effects ANOVAs with subject mean arousal ratings as the dependent measure, subject group as the between-subjects factor and stimulus type as the repeated-measures factor. Because the focus of the current study was on responses to images depicting nude females, nude males, and heterosexual couples engaged in sexual activity, the behavioral and imaging data

for images of same-sex couple sexual activity are not reported here. Imaging data were analyzed using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>) (Frackowiak, 2004). During preprocessing, overall data quality was initially checked using ArtRepair software (Mazaika et al., 2005), and all subjects were checked for adequate signal in the bilateral amygdala region. Next, differences in slice acquisition timing were corrected and images were realigned and unwarped to correct for motion-related artifacts. Normalization of functional images to the standard Montreal Neurological Institute (MNI) anatomical template was conducted using SPM8's unified segmentation and normalization method, followed 8 mm full-width half maximum (FWHM) smoothing (Friston et al., 1994).

Statistical post-processing of fMRI data used a standard two-stage mixed-effects approach. For individual subjects, activity for events corresponding to each of the six stimulus conditions was modeled with a train of delta functions representing onset and duration, convolved with a canonical hemodynamic response function. The inter-trial fixation period served as an implicit baseline. Activation contrasts between conditions (e.g., sexually arousing couples vs. neutral stimuli) were assessed using linear contrasts, and subject-specific contrast images were generated from linear combinations of the corresponding estimated beta-weights at each voxel in the brain. Group differences in activation were assessed with a second-level, mixed-effects analysis, using a set of two-group unpaired t-tests on the individual subject-specific contrast images, yielding statistical parametric maps (Josephs et al., 1997). Because women with CAIS were older, on average, than control men and women, age was included as a covariate in the analyses. Nevertheless, among women with CAIS, activations in the key regions of interest did not decrease significantly with increasing age, suggesting that between-group differences in activation could not be attributed to age differences.

Correction for multiple statistical comparisons was calculated using Monte-Carlo simulations using AlphaSim (http://afni.nimh.nih.gov/pub/dist/doc/program_help/AlphaSim.html), with an initial cluster-forming single-voxel threshold of $p < 0.005$ (uncorrected), and a grey matter brain mask (Berns et al., 2012), yielding a minimum cluster size of 837 mm^3 to achieve a whole-brain-corrected cluster-wise threshold of $p < 0.05$. For independent assessment of the a priori bilateral amygdala region, we computed the cluster-wise threshold using the anatomical volume of the bilateral amygdala (Tzourio-Mazoyer et al., 2002), yielding a combined cluster-forming single-voxel threshold of $p < 0.01$ and a minimum size of 270 mm^3 to reach a corrected threshold of $p < 0.05$.

Results

All three groups gave similar sexual arousal ratings for the couples engaged in heterosexual activity, and gave significantly lower arousal ratings (minimally above baseline) for the neutral couples (Fig. 1). A group (men, control women, women with CAIS) \times stimulus (arousing couples vs. neutral couples) ANOVA on sexual arousal ratings revealed an effect of stimulus, $F(1, 35) = 170.23$, $p < 0.001$, $\eta^2 = 0.82$, but no effect of group, $F(2, 35) = 1.69$, $p = 0.20$, $\eta^2 = 0.09$, and no interaction between group and stimulus, $F(2, 35) = 1.80$, $p = 0.18$, $\eta^2 = 0.02$. As expected, men gave higher sexual arousal ratings to nude female images than to nude male images (Fig. 1). Control women and women with CAIS showed less stimulus specificity in their arousal ratings, giving very similar (albeit not high) ratings of sexual arousal to both nude female and nude male images. A Group (men, control women, women with CAIS) \times Stimulus (nude females v. nude males) ANOVA on sexual arousal ratings revealed an effect of Stimulus, $F(1, 35) = 14.10$, $p < 0.001$, $\eta^2 = 0.17$, no effect of Group, $F(2, 35) = 0.46$, $p = 0.63$, $\eta^2 = 0.03$, and a Group \times Stimulus interaction, $F(2, 35) = 17.20$, $p < 0.001$, $\eta^2 = 0.41$.

To determine further whether women and women with CAIS showed less stimulus specificity when each group was compared

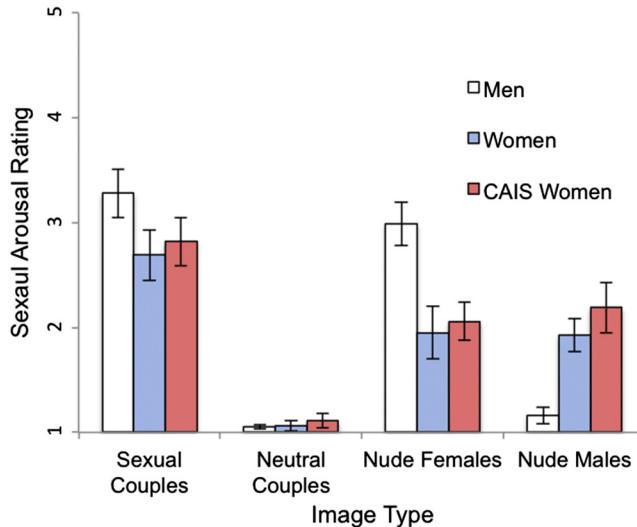


Fig. 1. Sexual arousal ratings for each type of image for men (white bars), control women (blue bars), and women with CAIS (red bars). Statistical comparisons for these ratings are presented in the [Results](#) section. Error bars indicate the standard error of the mean. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

separately to men (men vs. control women; men vs. women with CAIS), two additional one-way ANOVAs were conducted with the sexual arousal ratings for the preferred category minus the corresponding ratings for the non-preferred stimulus category for each group as the dependent measure (i.e., ratings for nude females minus ratings for nude males for men and the converse for women and women with CAIS). Both analyses confirmed that men showed greater differential ratings, indicating greater stimulus specificity, when compared to both control women (significant effect of Group, $F(1, 24) = 26.36$, $p < .001$, $\eta^2 = 0.53$), and women with CAIS (significant effect of Group, $F(1, 25) = 30.68$, $p < .001$, $\eta^2 = 0.56$). Post hoc paired t-tests comparing sexual arousal ratings between stimulus types for each group confirmed that men gave higher ratings for nude female than nude male images, $t(12) = 9.19$, $p < 0.001$, $d = 5.31$, whereas ratings did not differ by stimulus type for either control women, $t(11) = 0.09$, $p = 0.93$, $d = 0.05$, or women with CAIS, $t(12) = 0.45$, $p = 0.66$, $d = 0.26$ (Bonferroni corrected alpha = 0.017) (Fig. 1).

The neuroimaging results supported the prediction that fMRI responses in women with CAIS would differ from those of control men and would be similar to those of control women. In the first primary neuroimaging analysis, replicating prior findings (Hamann et al., 2004), men showed significantly greater right amygdala activation than did control women to the sexually arousing nude heterosexual-activity couple stimuli (vs. neutral-couple stimuli) (Fig. 2a). Men also showed significantly greater bilateral amygdala activation than women with CAIS (Fig. 2b). The clusters of greater right amygdala activation in both group contrasts overlapped spatially and had similar maximal loci (Table 1). There were no significant activation differences between women with CAIS and control women (Fig. 2c). Thus, results are consistent with the prediction that CAIS women would respond similarly to control women.

In the second primary analysis, we assessed the category-specific response profiles of heterosexual men and women to determine whether the brain responses of women with CAIS are more similar to those of heterosexual women or heterosexual men. To assess category-specificity we compared each group on fMRI responses to images from their preferred vs. nonpreferred category of sexual partners. As predicted, men showed a highly category-specific fMRI response, with greater activation to images of nude women than to images of nude men in several brain regions associated with sexual response and emotion (Fig. 3a, Table 2) (Safron et al., 2007). Men showed no regions of

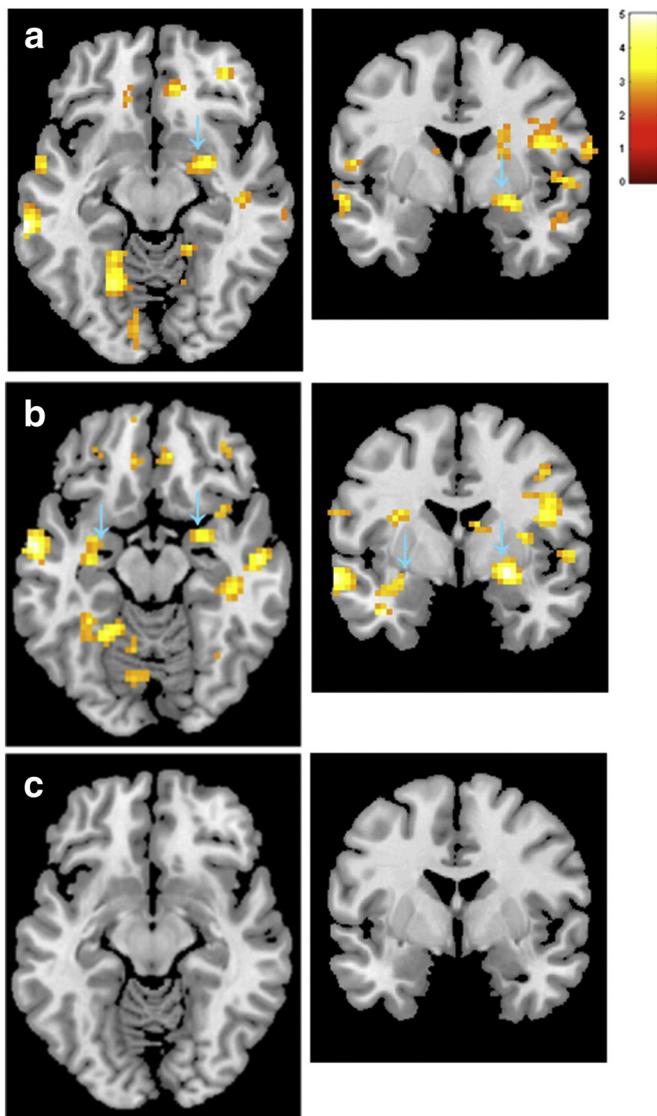


Fig. 2. Regional brain activation maps comparing activation elicited by sexual vs. neutral couples images for men, control women and women with CAIS ($p < 0.05$, corrected for multiple comparisons within the region of interest). Blue arrows indicate activation clusters that include the amygdala. (a) Greater right amygdala activation for men > control women (left panel: axial view at $z = -11$; right panel: coronal view at $y = -1$) (b) Greater left and right amygdala activation for men > women with CAIS (left panel: axial view at $z = -14$; right panel: coronal view at $y = -1$), (c) No differences in amygdala activation were observed between women with CAIS and control women. Color bar indicates maximal Z values for activation maps. The right hemisphere is on the right of the coronal images and bottom of the axial images. See Table 1 for a full listing of activations for these group contrasts. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

greater activation to images of nude men vs. women. Control women also showed a category-specific fMRI response to their preferred category (nude men vs. nude women), but as predicted based on reports of lower category-specificity in women, this differential response occurred across a considerably smaller and more limited set of regions, including both the left and right amygdala (Fig. 3b, Table 2). Like men, control women exhibited no regions of greater activation for their nonpreferred category.

Similar to control women, women with CAIS exhibited category-specific responses to nude men that were substantially smaller in magnitude and anatomical extent than the corresponding responses of control men to their preferred partner category. The differential responses in women with CAIS were seen in a limited set of ventral prefrontal regions and in the same left amygdala region found for

Table 1

Group differences in activation elicited by heterosexual couple stimuli > neutral couple stimuli.

Region	MNI coordinates					
	L/R	x	y	z	T	k
<i>Men > Women</i>						
Amygdala	R	27	−1	−11	3.78	19
Lingual g.	L	−21	−58	−8	4.78	159
Mid. temporal g.	R	45	−19	−14	4.07	44
Calcarine ctx.	R	9	−82	−2	3.98	39
Mid. frontal g.	L	−30	11	31	3.83	31
Cuneus	R	15	−97	10	3.61	37
Mid. frontal g.	R	51	44	19	3.32	40
<i>Women > men</i>						
No activation clusters						
<i>Men > women with CAIS</i>						
Amygdala	L	−30	−1	−14	3.09	12
Amygdala	R	24	−1	−11	4.71	21
Precentral g.	R	42	2	19	4.79	182
Putamen	R	24	−1	−8	4.77	83
Lingual g.	L	−24	−49	−11	4.71	127
Mid. temporal pole	L	−39	2	−29	4.62	70
Mid. temporal g.	L	−60	−4	−14	4.61	56
Med. orbital frontal g.	R	12	56	−5	3.82	53
Lingual g.	L	−12	−73	−8	3.42	41
<i>Women with CAIS > men</i>						
No activation clusters						
<i>Women with CAIS > women</i>						
No activation clusters						
<i>Women > women with CAIS</i>						
No activation clusters						

Note: Table lists coordinates of the activation maxima (in standard MNI space (Tzourio-Mazoyer et al., 2002)), maximal T statistics, and size (in voxels; k) for all significant clusters ($p < 0.05$, corrected for multiple comparisons across the brain). For the a priori amygdala region of interest, multiple-comparisons correction was based on the volume of the bilateral amygdala ($p < 0.05$). L = left hemisphere. R = right hemisphere.

control women (Table 2), regions associated with emotional and sexual responses (Stoléru et al., 2012). Notably, unlike either men or control women, women with CAIS showed two regions of greater activation to their nonpreferred stimulus category (nude women), in areas typically associated with visual perception (right occipital cortex) and executive function (right dorsolateral prefrontal cortex) rather than sexual response (Stoléru et al., 2012; Yarkoni et al., 2010).

We further assessed the spatial extent of category-specific responses in each group by comparing the number of activated voxels showing category-specific responses. By this measure, men showed category-specific responses with the greatest spatial extent, women showed category-specific responses in substantially fewer voxels (28% of the number of category-specific voxels in men), and women with CAIS showed the smallest number of category-specific voxels (3% of the total number for men, and 11% of the total number control women) (see Table 2 for the location of brain regions corresponding to the clusters of category-specific voxels).

Separate examination of responses of women and women with CAIS to images of nude women (vs. neutral stimuli) and to nude men (vs. neutral stimuli) indicated that their limited differential response resulted from substantial and highly similar activation responses to nude men and nude women (e.g., relative to neutral stimuli), rather than an overall weak activation response to the nude stimuli. For example, there was substantial spatial overlap between women and women with CAIS in their responses to their preferred stimulus type (nude males) vs. neutral stimuli, as assessed by the statistical overlap (conjunction) between the corresponding group activation maps (at $p < .05$, corrected for multiple comparisons in each group). The areas of common significant activation (conjunction) spanned a large spatial extent, comprising 5927 voxels, in 12 activation clusters that included multiple cortical and subcortical regions including orbitofrontal, middle, and superior prefrontal cortex, anterior and mid-cingulate cortex, insula, parietal cortex, middle and superior temporal cortex, middle and inferior occipital cortex, putamen, thalamus, hippocampus and amygdala, and the cerebellum. In contrast, the two groups significantly differed in response to their preferred stimulus type (nude males vs. neutral stimuli) in a much more limited and

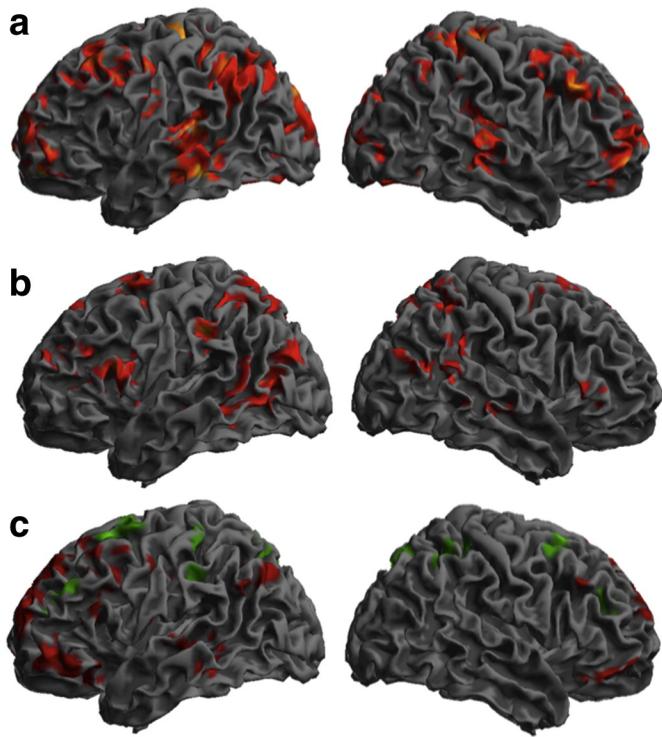


Fig. 3. Regional brain activation maps comparing activation elicited by nude female vs. nude male stimuli for men, control women and women with CAIS ($p < 0.05$, corrected for multiple comparisons). Activations are overlaid on canonical cortical surface maps of the left and right hemispheres, displayed on the left and right of the figure, respectively. (a) Control men: greater activations for preferred vs. non-preferred stimuli (nude female vs. nude male), (b) Control women: greater activations for preferred vs. non-preferred stimuli (nude male vs. nude female). (c) Combined map showing regions of greater activation for control men vs. women with CAIS for the contrast between men's preferred > non-preferred stimulus category (red regions) and regions of greater activation for control women vs. women with CAIS for the contrast between control women's preferred > non-preferred stimulus category (green regions). Activation responses of women with CAIS differed markedly from men's responses as evidenced by the large number/size of red regions associated with emotion and sexual response (Table 3), particularly on the right, and were highly similar to those of control women, as evidenced by the small number/size of green regions in superior prefrontal and parietal cortex not specifically associated with sexual arousal responses (Table 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

smaller set of regions, including a total of 311 voxels in 3 clusters in occipital and parietal cortex for the women > women with CAIS group contrast, and including 310 voxels in 2 clusters in medial prefrontal cortex and parietal cortex for the reverse group contrast (at $p < .05$, corrected for multiple comparisons).

These results for each group were further corroborated by between-group contrasts which found that brain responses of women with CAIS to sexual images were substantially more similar to those of heterosexual women than heterosexual men. To assess similarity, we compared groups on the corresponding preferred vs. nonpreferred category activation contrasts for heterosexual men and women. If the responses of women with CAIS are more similar to those of control women than control men, comparisons between women with CAIS and control women on heterosexual women's preferred category contrast should yield fewer differential activations than the corresponding comparison between women with CAIS and control men on heterosexual men's preferred category contrast. Consistent with this, control men exhibited markedly greater activation to nude female vs. male stimuli than did women with CAIS and control women, across a large set of regions including ventral prefrontal and inferior temporal regions associated with emotion and sexual response (Table 3, Fig. 3c). Conversely, for heterosexual women's preferred vs. nonpreferred category contrast (nude males > nude females), the group comparison between women

Table 2
Activations elicited by preferred vs. non-preferred sexual stimuli in each group.

Region	MNI coordinates					
	L/R	x	y	z	T	k
Men						
<i>Female > male stimulus contrast</i>						
Mid. & inf. temporal g.	*	−51	−34	−11	11.62	8191
Lingual g., fusiform g.						
Mid. cingulate g.						
Inf. orb. frontal g.,	*	−42	53	−14	6.84	1000
Sup. med. frontal g.						
Mid. & sup. frontal g.						
Sup. frontal g.	R	15	26	49	5.69	92
Sup. frontal g.	L	−15	38	37	5.03	42
Sup. orb. frontal g.	R	21	47	−17	4.04	40
<i>Male > female stimulus contrast</i>						
No activation clusters						
Women with CAIS						
<i>Male > female stimulus contrast</i>						
Amygdala	L	−18	5	−20	4.35	18
Inf. orb. frontal g.	L	−33	29	−20	5.27	47
Inf. orb. frontal g.	R	51	44	−17	4.93	44
Sup. med. frontal g.	L	−18	5	−20	3.97	33
<i>Female > male stimulus contrast</i>						
Occipital cortex	R	12	−76	1	5.07	86
Prefrontal cortex	R	30	38	28	5.02	47
Women						
<i>Male > female stimulus contrast</i>						
Amygdala	L	−21	−1	−20	4.56	55
Amygdala	R	21	2	−20	4.35	18
Precuneus	L	−9	−64	31	10.78	580
Mid. temporal g.	R	60	−34	−5	7.80	101
Inf. parietal g.	L	−54	−34	40	7.40	250
Mid. temporal g.	L	−39	−64	7	7.29	416
Inf. frontal oper.	L	−60	17	16	6.78	268
Sup. frontal g.	L	−27	2	67	6.77	170
Mid. frontal g.	R	27	−7	46	5.99	56
Sup. med. frontal g.	R	3	35	52	5.55	81
Mid. temporal g.	R	45	−58	16	5.25	178
Sup. frontal g.	R	18	29	61	5.09	74
Mid. frontal g.	L	−24	47	25	5.06	146
Inf. parietal g.	R	39	−52	52	4.78	103
Inf. frontal g.	R	57	23	4	4.56	55
Parahippocampal/amygdala	R	21	2	−20	4.56	50
<i>Female > male stimulus contrast</i>						
No activation clusters						

Note: Table lists coordinates of the activation maxima (in standard MNI space), maximal T statistics, and size (in voxels; k) for all significant clusters ($p < 0.05$, corrected for multiple comparisons across the brain). Multiple region summary labels are listed for very large activation clusters that spanned several brain regions. For the a priori amygdala region of interest, multiple-comparisons correction was based on the volume of the bilateral amygdala ($p < 0.05$). L = left hemisphere. R = right hemisphere. * = regions span both hemispheres.

and women with CAIS yielded few activation differences, with greater activation for control women observed in limited regions of the superior prefrontal and parietal cortex not specifically associated with sexual arousal responses (Table 3, Fig. 3c).

Discussion

These converging findings indicate that women with CAIS have brain responses to sexually arousing stimuli similar to those of control women, with whom they share female-typical socialization and predominantly estrogenic post-natal hormonal exposure. Their brain responses differed from those of control men with whom women with CAIS share 46,XY chromosome complement and presumed high prenatal and infant testosterone secretion to which women with CAIS could not respond because they lack androgen receptors. In all analyses, the fMRI responses of women with CAIS differed substantially from those of men, and were markedly similar to those of control women. Men showed greater amygdala activation than did both groups of women to images of nude male-female couples engaged in sexual activity. Category-specificity analyses of fMRI responses to male-preferred

Table 3

Group differences in activation elicited by female vs. male sexually arousing stimuli.

Region	MNI coordinates					
	L/R	x	y	z	T	k
Greater activation to female vs. male stimuli						
<i>Men > Women</i>						
Precentral g., mid. & inf. temporal, sup. parietal g., mid. & sup. prefrontal, mid. & ant. cingulate	*	24	–25	49	7.82	11,595
Mid. frontal g.	R	24	44	4	5.42	788
Thalamus	L	–6	–13	10	4.16	108
Caudate	R	15	11	16	4.02	39
Sup. orb. frontal	R	18	38	–17	3.90	31
Inf. orb. frontal	R	54	23	–5	3.68	45
Insula	L	–45	5	–6	3.55	42
<i>Women > men</i>						
<i>Men > women with CAIS</i>						
Bilat. Med. sup. frontal g., l. mid. frontal	*	–12	59	19	5.61	964
Inf. orb. frontal g.	R	36	32	–17	5.39	165
Inf. orb. frontal g.	L	–42	56	–11	5.24	309
Mid. temporal g.	L	–57	–37	–14	4.90	181
Lingual g.	R	33	–70	1	4.68	34
Cerebellum	L	–33	–73	–29	4.28	168
Med. orb. frontal g.	R	3	56	–11	4.24	85
Angular g.	L	–33	–73	40	4.15	149
Postcentral g.	L	–42	–13	40	4.10	32
Mid. frontal g.	R	54	23	34	4.10	49
Rolandic operculum	L	–45	–13	16	4.09	38
Inf. frontal trigone	L	–42	29	16	3.48	43
Lingual g.	L	–12	88	–2	3.39	44
Precuneus	L	–6	–49	34	3.26	34
<i>Women with CAIS > men</i>						
<i>No activation clusters</i>						
Greater activation to male vs. female stimuli						
<i>Women > women with CAIS</i>						
Mid. frontal g.	L	–12	2	70	5.13	187
Mid. frontal g.	L	–27	41	16	4.96	85
Sup. frontal g.	R	24	14	52	4.74	168
Inf. parietal g.	R	24	–34	52	4.71	136
Inf. parietal g.	L	–54	–34	40	4.69	65
Precuneus	R	9	–73	49	4.66	397
Mid. cingulate g.	L	–18	–40	43	4.44	64
Mid. frontal g.	L	–30	35	28	4.38	80
<i>Women with CAIS > women</i>						
<i>No activation clusters</i>						

Note: Table lists coordinates of the activation maxima (in standard MNI space) (Tzourio-Mazoyer et al., 2002), maximal T statistics, and size (in voxels; k) for all significant clusters ($p < 0.05$, corrected for multiple comparisons across the brain). Multiple region labels are listed for very large activation clusters that spanned several brain regions. L = left hemisphere. R = right hemisphere. * = regions span both hemispheres.

vs. female-preferred nude stimuli showed that men's category-specific responses differed markedly from those of both groups of women, whereas women with CAIS and control women showed highly similar responses.

Brain responses of women with CAIS, while similar to those of control women, did not match them in all respects, particularly in the second analysis, in which women with CAIS showed significantly less category-specificity than did control women. Because women in general show reduced category-specificity in sexual arousal responses to sexual stimuli (Chivers, 2005; Chivers and Bailey, 2005; Chivers et al., 2004), this reduced category-specificity may reflect a heightened female-typical response due to reduced or absent response to endogenous androgens compared with control women who are functionally exposed to higher levels of adrenal and ovarian androgens. Alternatively, this could reflect a feminizing effect of prenatal estrogens derived from testosterone, which women with CAIS would be expected to have substantially greater levels of than would control women.

We cannot say whether the difference between women with CAIS and control women reflects organizational or activational effects of androgens. Whatever the mechanism, the differences between women with CAIS and control women was much smaller than that between CAIS women or control women and men. The responses of the

three groups differed in magnitude similarly to their presumed degree of exposure, or response, to androgens, supporting the idea that the differences in category specificity and/or brain activity reflect organizational and/or activational effects of hormones beyond any influences of socialization.

In the absence of the capacity to respond to androgens, women with CAIS exhibited female-typical brain responses to sexual stimuli, indicating that possessing a Y chromosome is not sufficient for male-typical brain response. Similarly, these data argue against a role of the aromatization of testosterone to estrogens in the masculinization of the human brain (Balthazard and Ball, 2012). Women with CAIS produce significantly higher levels of testosterone than do control women and presumably produce comparably higher levels of estrogens derived from testosterone than do control women. Yet, women with CAIS showed no evidence of masculinization of their neural responses or evaluation of sexual stimuli despite this exposure to elevated aromatized estrogens, suggesting a central role for effective androgen exposure requiring functional androgen receptors in creating a male-typical response pattern. Consistent with this idea, category specificity varied with the presumptive degree of androgen exposure or action, with category-specificity highest in control men, lower in control women, and lowest in women with CAIS.

While the current results are consistent with reduced responsiveness to androgens and a lack of an effect of aromatized estrogens in women with CAIS resulting in a lack of masculinization of responses, we cannot definitively conclude that these results are a direct effect of reduced androgen effectiveness, because all women with CAIS were raised female and likely received female-typical socialization. However, our findings strongly suggest a causal role for limited responsiveness to androgens in women with CAIS resulting in feminine development despite the presence of a Y chromosome and high levels of testosterone and estrogens, providing novel evidence that 46,XY karyotype alone does not result in male-typical brain response in humans.

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