Sex differences in autism: a resting-state fMRI investigation of functional brain connectivity in males and females

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Abstract

Autism spectrum disorders (ASD) are far more prevalent in males than in females. Little is known however about the differential neural expression of ASD in males and females. We used a resting-state fMRI-dataset comprising 42 males/42 females with ASD and 75 male/75 female typical-controls to examine whether autism-related alterations in intrinsic functional connectivity are similar or different in males and females, and particularly whether alterations reflect 'neural masculinization', as predicted by the Extreme Male Brain theory. Males and females showed a differential neural expression of ASD, characterized by highly consistent patterns of hypo-connectivity in males with ASD (compared to typical males), and hyper-connectivity in females with ASD (compared to typical females). Interestingly, patterns of hyper-connectivity in females with ASD reflected a shift towards the (high) connectivity levels seen in typical males (neural masculinization), whereas patterns of hypo-connectivity observed in males with ASD reflected a shift towards the (low) typical feminine connectivity patterns (neural feminization). Our data support the notion that ASD is a disorder of sexual differentiation rather than a disorder characterized by masculinization in both genders. Future work is needed to identify underlying factors such as sex hormonal alterations that drive these sex-specific neural expressions of ASD.

Key words: autism spectrum disorders; sex differences; functional connectivity; resting-state fMRI

Introduction

Sex is increasingly recognized as a source of heterogeneity in psychiatric neuropathophysiology. Some disorders such as autism spectrum disorders (ASD) are far more prevalent in males compared to females, with sex ratios in the range of 3–4 males per female (Fombonne, 2005). For high-functioning autism, this ratio even increases to 6–8 males per female. The strong male-bias in ASD prevalence suggests that sex-specific biological factors may play a pivotal role in ASD etiology.

The 'Empathizing-Systemizing' (E-S) theory postulates that in the general population, normal sex differences exist in domains of empathy (the ability to identify oneself with other’s mental state and to respond to these feelings and thoughts with an appropriate response) and systemizing (the ability to analyze and build systems) with typical females exhibiting more empathizing and less systemizing compared to typical males (Baron-Cohen, 2009; Baron-Cohen et al., 2014). In relation to autism, the ‘Extreme Male Brain’ (EMB) theory extends this notion by proposing that people with ASD are characterized by an extreme form of the typical male E-S cognitive profile and ‘hyper-masculinization’ of the brain (Baron-Cohen, 2002; Baron-Cohen et al., 2011).

Strong cognitive-behavioral support for the E-S and EMB theory came from a recent study including 800 participants with...
ASD and 3.900 controls showing that on average, typical females scored higher on empathy, while typical males scored higher on the systematizing and autistic traits, and that both males and females with autism showed a shift toward the extreme of the “male profile” on these measures (Baron-Cohen et al., 2014). Sex differences in the typical population were also attenuated in males and females with ASD (Baron-Cohen et al., 2014), which is in line with a recent meta-analysis revealing only minor differences between ASD males and ASD females in the domain of social behavior and communication (Van Wijngaarden-Cremers et al., 2014). In the domain of repetitive and restricted behaviors on the other hand, reductions in females compared to males have been consistently reported (Mandy et al., 2012; Szatmari et al., 2012; Frazier et al., 2014; Van Wijngaarden-Cremers et al., 2014).

At the neurophysiological level, only few studies explored sex-specific expressions of ASD and revealed a complex relationship between autism and sex. In general, previous studies found that females with autism show a differential expression of ASD compared to males with autism in terms of early brain overgrowth (Sparks et al., 2002), brain structure (Beacher et al., 2012a; Lai et al., 2013) and neural recruitment during task-based fMRI (Beacher et al., 2012b; Schneider et al., 2013).

One neuroanatomical study demonstrated that typical sexual dimorphisms in typical controls were absent or attenuated in males and females with ASD (Beacher et al., 2012a), whereas a more recent study identified significant alterations in grey and white matter in both sexes, but with minimal spatial overlap in the implicated regions in females and males (Lai et al., 2013). Also results from two task-based fMRI studies are supportive of a differential sex-specific expression of ASD, as revealed by opposite diagnosis-related effects in the two sexes; with females showing ASD-related hypo-activation (compared to typical females), and males showing hyper-activations (compared to typical males) during a mental rotation task (Beacher et al., 2012b) and empathy tasks (Schneider et al., 2013). Interestingly, several of the aforementioned studies reported ‘neural masculinization’ of the brain, but these effects were most pronounced for females with ASD. Particularly, compared to typical females, females with ASD displayed alterations in several brain regions, and these changes reflected a shift towards the neuroanatomy (Lai et al., 2013) or brain activation patterns (Beacher et al., 2012b) seen in typical males. No such effect of ‘neural masculinization’ or ‘hyper-masculinization’ was observed in males with ASD. On the contrary, in several brain regions, males with ASD even showed opposite patterns, reflecting less masculinization or shifts towards a typical feminine brain profile.

Aside neural masculinization, an alternative but not mutually exclusive notion of ‘female-specific protective factors’ (FFP) has been put forward suggesting that females can withstand a larger etiological load than males before reaching diagnostic thresholds of ASD (Werling and Geschwind, 2013). At a genetic level, the FFP view is supported by some recent reports showing that females with ASD display an increased genetic load compared to affected males (Sebat et al., 2007; Levy et al., 2011; Sanders et al., 2011) and that relatives of affected females with ASD are at an increased risk for ASD compared to relatives of affected males (Hallmayer et al., 2011; Robinson et al., 2013). If the FFP model would also hold at the neural level, it would predict that more ‘severe’ brain changes in females are necessary for them to reach the point of clinical diagnosis. To date however, no clear neurophysiological evidence has emerged in support of this view, as most of the reported brain alterations in females with ASD were not more extensive or spatially transcending the regions that are reported to be affected in males with ASD. It should be noted however that despite these initial efforts, prior ASD neurophysiological research has been strongly characterized by an underrepresentation and often deliberate ‘exclusion-by-design’ of female participants. As a consequence, studies including females were often characterized by relatively small sample sizes, making a comprehensive evaluation of sex differences in the ASD neural phenotype difficult.

Here, sex-specific changes in intrinsic functional connectivity were analyzed using a multicenter resting-state fMRI dataset, comprising 42 males/42 females with ASD and 75 male/75 female typical controls of the Autism Brain Imaging Data Exchange repository (ABIDE). While the entire ABIDE repository consists of more than 1000 resting-state fMRI scans of ASD patients and controls, the majority of included data are from males with ASD (i.e. reflecting the higher prevalence of ASD in males). However, given the rarity of females with ASD, coordinated multi-site efforts such as ABIDE are highly valuable as they allow for initial resting-state fMRI investigations of females with ASD using considerably large samples.

The dysconnection model of autism links ASD to altered brain connectivity, rather than localized changes in brain activation (Just et al., 2004; Minshew and Keller, 2010; Vissers et al., 2012; Müller et al., 2011; Kana et al., 2014). In light of this model, the current study aimed to examine whether ASD-related alterations in intrinsic functional connectivity are similar or different in males and females with ASD, and particularly (i) whether alterations in functional connectivity are more severe in females compared to males (as predicted by the FFP model); and/or (ii) whether alterations reflect a shift toward neural masculinization (as predicted by the EMB theory). Finally, if masculinization is observed, a key question will be whether masculinization is expressed equally in females and males.

Distinct indices of functional circuitry were examined. Two hypothesis-driven connectivity measures were chosen based on prior reports of diagnosis-related alterations in males with ASD, which included (i) investigations of the functional circuitry of the posterior superior temporal sulcus (STS), a key ‘hub’ in social information processing by connecting distinct social brain networks underlying theory of mind (amygdala-orbitofrontal network) and action/emotion understanding (fronto-parietal action perception network or mirror neuron system) (Lahnakoski et al., 2012); and (ii) exploration of functional connections of the posterior cingulate cortex (PCC), a core region of the default network (Buckner et al., 2008), most prominently studied in prior ASD resting-state neuroimaging research. ASD-related alterations in functional circuitry have been reported for both regions in males with ASD (STS (Shih et al., 2010; Kana et al., 2014; Mueller et al., 2013; Alaerts et al., 2014); PCC (Cherkassky et al., 2006; Monk et al., 2009; Assaf et al., 2010; Weng et al., 2010)), and a key question will be whether and how these alterations are different in females with ASD.

In addition to the hypothesis-driven investigations of STS and PCC circuitry, we further explored whether similar diagnosis- and/or sex-related patterns would also emerge based on more exploratory whole-brain connectivity analyses or whether patterns are specific to the a-priori chosen seed-based networks. To do so, whole-brain region-to-region functional connectivity was explored within a whole-brain parcellated network comprising 200 regions-of-interest (Cradock et al., 2012). This exploratory ‘whole-brain’ connectivity examination allowed an unbiased investigation of sex-specific connectivity changes in ASD.
Methods

Participants

To explore sex-related differences in functional connectivity, we used resting-state fMRI data from males and females included in the Autism Brain Imaging Data Exchange repository (ABIDE) (Di et al., 2014). ABIDE consists of 1112 datasets including 474 males with ASD, 65 females with ASD, 474 typical control (TC) males and 99 TC females.

Inclusion criteria for female participants were individuals (i) aged between 7 and 30 years, which represents approximately two standard deviations from the overall mean age across all female participants (15.7 ± 7.0 years); (ii) with a mean frame-wise displacement (FD) (head micro-movements) lower than 0.5 mm (Power et al., 2012); and (iii) with successful preprocessing. Inclusion criteria for sites were a total of at least three females with ASD and three typical females meeting the other inclusion criteria. These criteria yielded resting-state fMRI datasets of 42 females with ASD and 75 TC females (Table 1). Within each site, male participants (42 ASD, 75 TC) were selected to match the female participants pair-wise for age and IQ (Table 1).

All participants with ASD had a clinician’s DSM-IV-TR diagnosis of Autistic Disorder, Asperger’s Disorder, or Pervasive Developmental Disorder Not-Otherwise-Specified. The majority of subjects were additionally assessed using the Autism Diagnostic Observation Schedule (ADOS) modules 3 or 4 (Lord et al., 1999), the Autism Diagnostic Interview–Revised (Lord et al., 2004), or both (Table 1). The Social Responsiveness Scale (SRS)-Child Version or SRS-Adult Version was assessed for a subset of ASD and TC participants (Constantino et al., 2004).

Data acquisition

Data acquisition parameters varied across sites. Details on scan parameter and site-specific protocols are available at fcon_1000.projects.nitrc.org/indi/abide/.

Image preprocessing

SPM-8 (Wellcome Department of Imaging Neuroscience, London, UK) and the CONN functional connectivity toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012; http://www.nitrc.org/projects/conn) were used for image preprocessing and statistical analyses implemented in Matlab R2008a (Mathworks).

Resting-state fMRI images were spatially realigned, corrected for differences in slice acquisition time by temporal interpolation to the middle slice (reference = 17), normalized to the standard EPI-template of the Montreal Neurological Institute (MNI-152), resampled into 3-mm isotropic voxels and spatially smoothed with an isotropic 5-mm full-width-at-half-maximum Gaussian kernel.

Realignment parameters were modeled as regressors of no-interest and white matter and cerebrospinal fluid were removed as confounds following the implemented CompCor-strategy (Behzadi et al., 2007) in the CONN functional connectivity toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012). Residual time-series of the resting-state images were then band-pass filtered (0.009 < f < 0.08 Hz). No global signal regression was applied.

Given the potential confounding effects of micro-movements on resting-state functional connectivity (Power et al., 2012; Van Dijk et al., 2012), we examined group differences in mean frame-wise displacement (mean FD) (Table 1 and Supplementary Figure S1). Sex differences in mean FD were observed in the TC group (greater mean FD in males compared to females [t(148)=2.56, P < 0.05]), not in the ASD group [t(82)=1.32, P = 0.19]. Irrespective of sex, the ASD group showed greater mean FD than TC [t(232)=4.42, P < 0.001] (Supplementary Figure S1). We accounted for inter-individual differences in micro-movements by including mean FD-scores as a nuisance covariate at the group-level in all analyses. Furthermore, all reported analyses were performed on ‘scrubbed’ data (Power et al., 2012), i.e. censoring frames displaying FD > 0.5 mm or frame-wise changes in brain image intensity exceeding >0.5 %BOLD.

No sex differences in the percentage of scrubbed frames were observed in the ASD group [t(82)=1.11, P = 0.27] (Supplementary Figure S2A). In the TC group, a tentative sex difference was observed [t(148)=1.90, P = 0.06] indicating a tentatively higher percentage of scrubbed frames in males compared to females. Irrespective of sex, the ASD group showed a higher percentage of scrubbed frames compared to the TC group [t(232)=3.82, P < 0.001] (Supplementary Figure S2A).

Connectivity analyses

Distinct connectivity analyses were performed: (i) seed-to-voxel functional connectivity analysis of the posterior superior temporal sulcus (STS) (right and left); (ii) seed-to-voxel functional connectivity analysis of the posterior cingulate cortex (PCC); and (iii) a whole-brain region-to-region functional connectivity analysis, comprising 200 regions-of-interest (ROIs).

For all indices of functional circuitry, a factorial design was used to identify main effects of diagnosis (ASD, TC), sex (male, female) and diagnosis-by-sex interaction effects.

Hypothesis-driven seed-to-voxel functional connectivity analysis

Seed-to-voxel analyses were performed using 10 mm radius spheres positioned in the right and left STS, centered around MNI-coordinates [±47,−60,4] and in the PCC, centered around MNI-coordinates [−6,−52,40]. The FCC forms a major hub of the default mode network and coordinates were based on (Fox et al., 2005). The STS coordinates were based on (Alaerts et al., 2014) and represent the STS region (right hemisphere) that was activated during an emotional processing task employing point light displays in young male adults with ASD and TC. The same STS seed previously exhibited reduced functional connectivity in males with ASD relative to TC (Alaerts et al., 2014).

For each participant, we extracted the mean time-series by averaging across all voxels in each seed-region. We then computed bivariate correlation coefficients between the seed time-course and that of every other voxel in the brain. The resultant participant-level whole-brain correlation maps were Fisher z-transformed to z-value maps. All group-level analyses were carried out including four nuisance regressors (site, mean FD, Full-scale IQ and age), using a cluster-forming voxel-level height threshold of P < 0.01 (corresponding to t > 2.34), a cluster-wise spatial extent threshold at P < 0.05, family-wise-error (FDR) corrected for multiple comparisons and with the minimal number of voxels in each cluster set at 100 voxels.

Exploratory whole-brain ROI-to-ROI functional connectivity analysis

Exploratory whole-brain ROI-to-ROI functional connectivity analyses were performed for a whole-brain parcellation generated via spatially constrained spectral clustering, comprising 200 ROIs (Craddock et al., 2012). For each participant, we
### Table 1. Group characteristics for the included male and female participants, separately for the ASD and TC groups

|                            | ASD Male–female | TC Male–female | Sex differences | ASD Male–female | TC Male–female | Sex differences | Group differences |
|-----------------------------|-----------------|----------------|-----------------|-----------------|----------------|-----------------|--------------------|
|                            | Males (Total n = 42) | Females (Total n = 42) | Sex differences | Males (Total n = 75) | Females (Total n = 75) | Sex differences | t-value | p     | t-value | p     | t-value | p     |
| Age at scan (years)        | 42 12.99 3.05 | 42 13.30 3.91 | 0.41 0.68 0.00 | 75 13.70 4.64 | 75 13.86 4.69 | 0.21 0.83 | 1.09 0.28 |
| Full IQ                    | 42 101.34 15.17 | 42 101.36 14.60 | 0.00 1.00 | 75 111.24 10.90 | 75 110.34 13.64 | 0.45 0.66 | 5.22 <.0001 |
| Verbal IQ                  | 42 103.95 14.51 | 42 101.64 16.98 | 0.67 0.50 | 75 112.28 12.15 | 75 110.89 14.65 | 0.63 0.53 | 4.51 <.0001 |
| Performance IQ             | 42 102.45 14.23 | 42 101.24 15.39 | 0.38 0.71 | 75 108.99 12.48 | 75 107.92 13.73 | 0.50 0.62 | 3.54 <.0001 |
| Head Motion (mean FD)      | 42 0.22 0.13 | 42 0.19 0.10 | 1.32 0.19 | 75 0.16 0.09 | 75 0.13 0.07 | 2.56 0.05 | 4.42 <.0001 |
| ADI-R                      | Social 38 20.08 5.50 | 39 19.72 5.84 | 0.28 0.78 | 0 | 0 | 0 |
|                            | Verbal 38 15.58 3.98 | 39 15.81 5.07 | -0.22 0.83 | 0 | 0 | 0 |
|                            | RRB 38 6.24 2.76 | 39 5.70 2.28 | 0.93 0.35 | 0 | 0 | 0 |
| ADOS Scores                | Total Score 25 12.56 3.73 | 25 10.66 3.28 | 1.92 0.06 | 0 | 0 | 0 |
|                            | Social 25 3.88 1.42 | 25 3.17 1.22 | 1.90 0.06 | 0 | 0 | 0 |
|                            | Communication 25 8.68 2.51 | 25 7.49 2.45 | 1.70 0.10 | 0 | 0 | 0 |
|                            | RRB 21 2.67 1.83 | 20 1.80 1.67 | 1.58 0.12 | 0 | 0 | 0 |
| SRS Raw Total Scores       | 18 93.67 24.78 | 19 90.11 30.49 | 0.39 0.70 | 31 16.65 13.81 | 31 22.26 14.32 | -1.57 0.12 | 17.23 <.0001 |

ASD, autism spectrum disorder; TC, typical controls; IQ, intelligence quotient; mean FD, mean framewise displacement; ADI-R, Autism Diagnostic Interview-Revised; ADOS, Autism Diagnostic Observation Schedule; RRB, Restricted Repetitive Behaviors; SRS, Social Responsiveness Scale; SD, standard deviation.

Male and female participants were included from 8 sites: (1) Kennedy Krieger Institute; (2) NYU Langone Medical Center; (3) Stanford University; (4) University of California, Los Angeles—Sample 1; (5) University of Leuven—Sample 2; (6) University of Michigan—Sample 1; (7) University of Pittsburgh, School of Medicine; (8) Yale Child Study Center. Detailed information on the diagnostic protocols at each site are publicly available on the ABIDE website (fcon_1000.projects.nitrc.org/indi/abide/). Males and females with and without ASD were matched for age, full-scale IQ, verbal IQ and performance IQ. Diagnostic groups (ASD, TC) were matched on age but not on IQ.
extracted the mean time series by averaging across all voxels in each ROI. We then computed bivariate correlation coefficients for each pair of ROIs. The resultant ROI-to-ROI correlation values were Fisher z-transformed. Group-level analyses on the ROI-to-ROI correlation matrices were carried out including four nuisance regressors (site, mean FD, Full-scale IQ and age). For completeness, ROI-to-ROI correlations are reported at an uncorrected $P < 0.001$ threshold, with a * indicating significant connections at a $P < 0.05$ FDR corrected threshold.

**Results**

**Seed-to-voxel functional connectivity**

**Diagnosis effect.** Main effects of diagnosis were explored separately for males and females and a conjunction analysis was performed to explore whether diagnosis effects demonstrated in males overlapped with those demonstrated in females.

**Males.** Overall, a predominant pattern of hypo-connectivity (ASD < TC) was revealed in males with ASD. Particularly, compared to typical males, males with ASD displayed hypo-connectivity with bilateral fusiform gyrus (FG) and left thalamus for the right STS-seed (3 clusters, see Figure 1A, Supplementary Table S1); and with bilateral inferior parietal cortex (IPL)-BA 6 and right insula for the left STS-seed (2 clusters, see Figure 2A, Supplementary Table S2). The PCC-seed showed hypo-connectivity with right superior frontal gyrus (SFG)/superior medial gyrus (SMG), bilateral thalamus and right angular gyrus/superior parietal lobule (SPL) (3 clusters, see Figure 3A, Supplementary Table S3). Only one cluster showed hyper-connectivity (ASD > TC) between the PCC-seed and right IPC/BA 6 (1 cluster, see Figure 3A, Supplementary Table S3).

**Females.** Overall, a predominant pattern of hyper-connectivity (ASD > TC) was revealed in females with ASD. Compared to typical females, females with ASD displayed hyper-connectivity with the right SFG/SMG and right inferior frontal gyrus (IFG) for the right STS-seed (2 clusters, see Figure 1A, Supplementary Table S1); and with mid cingulum, left parahippocampal gyrus, right SFG/SMG and cerebellum for the left STS-seed (4 clusters, see Figure 2A, Supplementary Table S2). The PCC-seed showed hyper-connectivity with right SFG/middle frontal gyrus (MFG), left MFG/BA 44 and right middle orbital gyrus (3 clusters, see Figure 3A, Supplementary Table S3). No clusters were identified that showed hypo-connectivity (ASD < TC) in females with ASD.

A conjunction analysis, exploring whether diagnosis effects demonstrated for males overlapped with those demonstrated for females, yielded no significant clusters. Only at a more lenient voxel-wise threshold of $P < 0.05$ and at an uncorrected $P < 0.05$ cluster-wise threshold, we identified one cluster in right SMG that showed hypo-connectivity (ASD < TC) with the PCC-seed both in males and females. This cluster partly overlapped with the cluster shown in Figure 3A.

**Sex effect.** Main effects of sex were explored separately for the ASD- and TC-groups and a conjunction analysis was performed to explore whether sex effects demonstrated for the ASD group overlapped with sex effects demonstrated in the TC group.

**ASD.** Overall, a predominant pattern of higher connectivity in females with ASD compared to males with ASD was revealed. For the left STS-seed, females with ASD showed higher connectivity than males with ASD with right SFG, and left IPC/BA 6 for the left STS-seed (2 clusters, see Figure 2B, Supplementary Table S2). No clusters were identified for the right STS-seed (Figure 1B). For the PCC-seed, ASD-females showed higher connectivity than ASD-males with right MFG/SFG and right MTG (2 clusters, see Figure 3B, Supplementary Table S3). No clusters were identified that showed higher connectivity in males compared to females with ASD.

**TC.** Overall, a predominant pattern of higher connectivity in typical males compared to typical females was revealed. Particularly, typical males showed higher connectivity than typical females with precuneus/mid cingulum for the left STS-seed (1 cluster, see Figure 2B, Supplementary Table S2). For the PCC-seed, typical males showed higher connectivity than typical females with left MFG/IFG (BA 44-45), and left superior temporal gyrus (STG)/MTG (2 clusters, see Figure 3B, Supplementary Table S2). No clusters were identified that showed higher connectivity in TC-females compared to TC-males.

A conjunction analysis, exploring whether sex effects demonstrated in the ASD group overlapped with those demonstrated in the TC group, yielded no significant clusters.

**Diagnosis-by-sex interaction.** Diagnosis-by-sex interaction effects were identified in one cluster for the right STS-seed, five clusters for the left STS-seed and two clusters for the PCC-seed. The observed pattern further extended findings of hyper-connectivity in females with ASD and hypo-connectivity in males with ASD. Particularly, females with ASD displayed hyper-connectivity compared to typical females, thereby reflecting a shift towards the (high) level of connectivity seen in typical males (Figures 1–3C). Males with ASD on the other hand, displayed hypo-connectivity compared to typical males, thereby reflecting a shift towards the (low) typical feminine connectivity pattern (Figures 1–3C, Supplementary Tables S1–S3). As indicated in the figure legends, several of the clusters showing diagnosis-by-sex interactions overlapped with clusters previously identified to show main diagnosis-related and/or sex effects (Figures 1–3C).

**Whole-brain ROI-to-ROI functional connectivity**

In addition to the hypothesis-driven investigations of STS and PCC functional circuitry, we also performed an ‘exploratory’ whole-brain functional connectivity examination within a whole-brain parcelled network, comprising 200 regions-of-interest.

**Diagnosis effect.** Compared to typical males, males with ASD displayed predominantly hypo-connectivity (blue connections in Figure 4A, left), whereas females with ASD displayed predominantly hyper-connectivity compared to typical females (red connections in Figure 4A, right). All affected connections are listed in Supplementary Table S4. In none of the identified connections, diagnosis effects (ASD < TC or ASD > TC) overlapped for males and females.

**Sex effect.** Females with ASD displayed predominantly higher connectivity than males with ASD (blue connections in Figure 4B, left; Supplementary Table S4), whereas typical females displayed predominantly lower connectivity compared to typical males (red connections in Figure 4B, right; Supplementary Table S4). For one connection, a similar sex effect was identified in the ASD and TC groups, indicating higher connectivity in females compared to males between left supramarginal gyrus (BA 41) and right insula (BA 13) (Supplementary Table S4).
Fig. 1. Seed-to-voxel connectivity of the right superior temporal sulcus (STS). (A) Clusters for which a main effect of diagnosis was revealed, separately for males (left) and females (right). Clusters showing hypo-connectivity (ASD < TC) are shown in blue; clusters showing hyper-connectivity (ASD > TC) are shown in red-yellow. As shown in (B), no significant main effect of sex was revealed for the right STS seed. (C) Clusters for which a diagnosis-by-sex interaction effect was revealed (left). The line plots (right) show functional connectivity scores as a function of diagnostic group (filled circles, ASD; open circles, TC), separately for males (grey lines) and females (pink lines). The thalamus cluster exhibited a diagnosis-by-sex interaction but also a diagnosis main effect in males (panel A, left). L, left hemisphere; R, right hemisphere; FG, fusiform gyrus; IFG, inferior frontal gyrus; SFG, superior frontal gyrus; SMG, superior medial gyrus. Vertical lines in the line plots denote SEM. Clusters are reported at a P < 0.05, FDR-corrected cluster-wise threshold. Significant clusters are overlaid on inflated surface maps generated using BrainNet Viewer (http://www.nitrc.org/projects/bnv/). Clusters are also listed in Supplementary Table 1.
Fig. 2. Seed-to-voxel connectivity of the left superior temporal sulcus (STS). (A) Clusters for which a main effect of diagnosis was revealed, separately for males (left) and females (right). Clusters showing hypo-connectivity (ASD < TC) are shown in blue; clusters showing hyper-connectivity (ASD > TC) are shown in red-yellow. (B) Clusters for which a main effect of sex was revealed, separately for ASD (left) and TC groups (right). Clusters showing higher connectivity in males are shown in brown; clusters showing higher connectivity in females are shown in pink-orange. (C) Clusters for which a diagnosis-by-sex interaction effect was revealed (left). The line plots (right) show functional connectivity scores as a function of diagnostic group (filled circles, ASD; open circles, TC), separately for males (grey lines) and females (pink lines). The left BA 6 cluster exhibits a diagnosis-by-sex interaction but also a diagnosis effect in males (panel A, left) and a sex main effect in the ASD (panel B, left). Clusters showing a diagnosis-by-sex interaction in the parahippocampal gyrus/thalamus, mid cingulum and right SFG/SMG overlapped with clusters identified to show a diagnosis effect in females (panel A, right) and sex effects either in the ASD group (panel B, left, for the right SFG/SMG cluster) or in the TC group (panel B, right, for mid cingulum cluster). L, left hemisphere; R, right hemisphere; BA, Brodmann area; IPC, inferior parietal cortex; SFG, superior frontal gyrus; SMG, superior medial gyrus. Vertical lines in the line plots denote SEM. Clusters are reported at a p < 0.05, FDR-corrected cluster-wise threshold. Significant clusters are overlaid on inflated surface maps generated using BrainNet Viewer (http://www.nitrc.org/projects/bnv/). Clusters are also listed in Supplementary Table S2.
Fig. 3. Seed-to-voxel connectivity of the posterior cingulate cortex (PCC). (A) Clusters for which a main effect of diagnosis was revealed, separately for males (left) and females (right). Clusters showing hypo-connectivity (ASD < TC) are shown in blue; clusters showing hyper-connectivity (ASD > TC) are shown in red-yellow. (B) Clusters for which a main effect of sex was revealed, separately for ASD (left) and TC groups (right). Clusters showing higher connectivity in males are shown in brown; clusters showing higher connectivity in females are shown in pink-orange. (C) Clusters for which a diagnosis-by-sex interaction effect was revealed (left). The line plots (right) show functional connectivity scores as a function of diagnostic group (filled circles, ASD; open circles, TC), separately for males (grey lines) and females (pink lines). The right SFG/SMG/MFG and left BA 6/BA 44 clusters showing a diagnosis-by-sex interaction overlap with clusters identified to show a diagnosis effect in females (panel A, right) and a sex effect in the ASD group (panel B, left). L, left hemisphere; R, right hemisphere; BA, Brodmann area; MTG, middle temporal gyrus; SFG, superior frontal gyrus; SMG, superior medial gyrus; MFG, middle frontal gyrus; IFG, inferior frontal gyrus; STG, superior temporal gyrus. Vertical lines in the line plots denote SEM. Clusters are reported at a p < 0.05, FDR-corrected cluster-wise threshold. Significant clusters are overlaid on inflated surface maps generated using BrainNet Viewer (http://www.nitrc.org/projects/bnv/). Clusters are also listed in Supplementary Table S3.
Diagnosis-by-sex interaction. Diagnosis-by-sex interaction effects further extended findings of hyper-connectivity in females with ASD and hypo-connectivity in males with ASD. Diagnosis-by-sex interaction effects were identified in 16 connections. In 14 of these connections, the diagnosis-by-sex interaction indicated that females with ASD displayed hyper-connectivity compared to typical females, thereby reflecting a shift towards the level of connectivity seen in typical males (red interaction pattern in Figure 4C). Within the same connections, males with ASD displayed hypo-connectivity compared to typical males, thereby reflecting a shift towards the typical feminine connectivity pattern (red interaction pattern in Figure 4C). This interaction pattern was identified in the majority of connections as listed in Table 2. The opposite pattern (blue interaction in Figure 4C), was only identified in two connections (Table 2).

The diagnosis-by-sex interaction revealed for the right SFG-left MTG connection and right SFG-precuneus/PCC connection (indicated in bold in Table 2) are consistent with diagnosis-by-sex interaction effects identified in the seed-to-voxel analyses from the left STS- and PCC-seed to right SFG (Figures 2C, 3C).

Relationship with symptom severity in the ASD group

Although not the primary aim of this study, we performed brain-behavior correlation analyses to explore whether the observed patterns of hypo-connectivity in males/hyper-connectivity in females with ASD was predictive of symptom severity as assessed by raw scores of the Social Responsiveness Scale (SRS) (Constantino et al., 2004) and ADOS total and subscale scores (social interaction, communication, restricted and repetitive behaviors) (Lord et al., 1999). To do so, correlations were calculated with connectivity scores ($z$-transformed $r$-values) from all clusters/connections that displayed diagnosis-by-sex interaction effects: one cluster for the right STS-seed, five clusters for the left STS-seed, two clusters for the PCC-seed (seen in Figures 1–3C) and 16 ROI-to-ROI connections (listed in Table 2). All brain-behavior relationships were calculated separately for males and females with ASD. We additionally explored whether the direction of the brain-behavior relationships was significantly modulated by sex.

SRS scores showed no significant positive or negative relationships with any of the connectivity measures after correction for multiple comparisons (Supplementary Figure S3A). Only at an uncorrected $P < 0.05$ threshold, males with ASD displayed a negative brain-behavior relationship in one connection (high ADOS scores correspond to low connectivity values), whereas in females with ASD, two connections showed a positive brain-behavior relationship (high ADOS scores correspond to high connectivity values) (Supplementary Figure S3A). Across connectivity measures, a tendency was revealed towards opposite brain-behavior relationships in males and females (negative brain-behavior relationship in males; positive brain-behavior relationship in females) (Supplementary Figure S3A, right). However, this ‘sex’-by-‘SRS’ interaction failed to reach significance ($F(1,33) = 2.33, P = 0.12$), which likely relates to the limited number of participants for which SRS scores were available (18 ASD males, 19 ASD females).

Also for the ADOS scores, none of the connectivity measures displayed significant positive or negative brain-behavior relationships after correction for multiple comparisons (Supplementary Figure S3B). Only at an uncorrected $P < 0.05$ threshold, one connection displayed a negative brain-behavior relationship in males, and one connection showed a positive brain-behavior relationship in females (Supplementary Figure S3B). Across connectivity scores, there was no tendency towards opposite brain-behavior relationships in males and females for any of the ADOS (sub)-scores.

Secondary analyses

Correction for head motion—scrubbed frames. Secondary analyses were performed to determine the extent to which our main findings of diagnosis-by-sex interaction effects are replicated in a sample excluding participants that displayed more than 20% of scrubbed frames (11 ASD-males, 8 ASD females, 10 TC-males, 5 TC-females). As seen in Supplementary Figure S2B, diagnosis-by-sex interaction effects remained significant for all clusters identified in the primary seed-to-voxel analyses. Also for the whole-brain ROI-to-ROI analysis, secondary analyses showed similar results (diagnosis-by-sex interaction patterns remained significant in 11 of the 16 connections identified in the primary analysis).

Whole-brain ROI-to-ROI functional connectivity using a different ROI template. In the primary analyses, diagnosis-by-sex interaction effects in ROI-to-ROI functional connectivity were explored using a whole-brain parcellated network of 200 ROIs (Craddock et al., 2012). In a secondary analysis, we explored whether similar diagnosis-by-sex interaction effects can be identified using a whole-brain ROI-to-ROI network comprising of 132 ROIs based on the FSL Harvard-Oxford Atlas (maximum likelihood cortical (91 ROIs)/subcortical atlas (15 ROIs)) and a cerebellar parcellation from the AAL Atlas (26 ROIs). As shown in Supplementary Figure S4, a similar pattern of diagnosis-by-sex interaction effects was revealed, indicating that females with ASD displayed hyper-connectivity compared to typical females, whereas males with ASD displayed hypo-connectivity compared to typical males.

Exploration of diagnosis-by-sex interaction effects within different age groups. In the primary analyses, diagnosis-by-sex interaction effects were identified across a wide age range (7–30 years). Although not the primary aim of this study, we verified whether the interaction effects were modulated by age. To do so, a secondary analysis was performed exploring diagnosis-by-sex interaction effects in three different age groups: childhood (7–12 years), adolescence (13–17 years) and adulthood (18–30 years). As seen in Supplementary Figure S5, a similar pattern of diagnosis-by-sex interaction effects was identified in each age group for all clusters identified in the primary seed-to-voxel analyses (three-way ‘age group’-by-diagnosis-by-sex interaction was not significant: $F(2, 219) = 0.22; P = 0.80$). Also for the 16 ROI-to-ROI connections identified from the whole-brain ROI-to-ROI connectivity analysis (listed in Table 2), the diagnosis-by-sex interaction effect was not significantly modulated by age group (three-way interaction: $F(2, 219) = 0.03; P = 0.90$) (data not shown).

Discussion

In this study, resting-state fMRI datasets of the ABIDE repository were used to examine whether ASD-related alterations in intrinsic functional connectivity are similar or different in males and females with ASD. A principal aim was to explore whether alterations fit predictions of the ‘Extreme Male Brain’ theory (EMB) (Baron-Cohen, 2002) by reflecting neural masculinization in males and/or females with ASD.
Fig. 4. Region-to-region functional connectivity within a whole-brain parcellation comprising of 200 regions of interest (ROIs). (A) Connections for which a main effect of diagnosis was revealed, separately for males (left) and females (right). Connections showing hypo-connectivity (ASD < TC) are shown in blue; connections showing hyper-connectivity (ASD > TC) are shown in red. (B) Connections for which a main effect of sex was revealed, separately for ASD (left) and TC groups (right). Connections showing higher connectivity in females are shown in blue; clusters showing higher connectivity in males are shown in red. (C) Connections for which a diagnosis-by-sex interaction effect was revealed (left). The line plots (right) show the identified interaction patterns as a function of diagnostic group (filled circles, ASD; open circles, TC), and sex (grey lines, males; pink lines, females). For completeness, ROI-to-ROI connections are reported at an uncorrected p < 0.001 threshold. L, left hemisphere; R, right hemisphere. Connections shown in panel A and B are listed in Supplementary Table S4. Connections shown in panel C are listed in Table 2.
Overall, our results showed that ASD-related alterations in functional connectivity were substantially different for males and females with ASD. Particularly, a number of clusters and connections were identified in which diagnosis-related differences in functional connectivity were in opposite direction, indicating that males with ASD generally exhibited hypo-connectivity whilst females with ASD generally exhibited hyper-connectivity relative to sex-matched typical controls. Even at more lenient statistical thresholds, we identified no regions for which diagnosis-related changes in connectivity (hypo- or hyper-connectivity) were in the same direction in males and females.

Interestingly, the general patterns of hyper-connectivity seen in females with ASD reflected a shift towards the (high) level of connectivity seen in typical males. Patterns of hypo-connectivity observed in males with ASD on the other hand, largely reflected a shift towards the (low) level of connectivity seen in typical females. Therefore, only in females with ASD, connectivity alterations were consistent with patterns of neural masculinization as predicted by the EMB theory (Baron-Cohen, 2002), whereas in males with ASD, connectivity alterations were consistent with a shift towards the typical feminine brain profile (neural feminization). Similar neural masculinization effects in females with ASD and less masculinization or shifts towards a typical feminine brain profile in males with ASD have been reported before in terms of neuroanatomy (Lai et al., 2013) and task-based brain activity during a mental rotation task (Beacher et al., 2012b). Here, we provide first evidence of neural masculinization/feminization patterns in intrinsic ‘resting-state’ functional connectivity in females and males with ASD respectively. These findings provide complementary support to the notion that autism may constitute a disorder of sexual differentiation or androgyny (as first suggested in Bejerot et al., 2012) rather than a disorder characterized by masculinization in both genders. In our study, sexual differentiation in the typical population was mostly reflected by patterns of lower connectivity in females compared to males (right B panel in all figures). However, in the ASD group, this pattern was reversed such that autistic females predominantly displayed higher connectivity compared to the autistic males (left B panel in all figures). These effects were further confirmed by the diagnosis-by-sex interaction analyses, showing that in the ASD group, reversed sexual differentiation patterns are found in brain regions that show typical sexual differentiation (C panel in all figures). Prior support for altered sexual differentiation in ASD came from a study by Bejerot et al. (2012) showing that females with ASD display more masculinized biological and physiological features than typical females, but that males with ASD display less masculine characteristics than males without ASD (Bejerot et al., 2012). Overall, it can be hypothesized that distinct biological/endo-crine mechanisms may drive the altered sexual differentiation in ASD. Findings of correlations between sex steroid levels such as fetal testosterone and measures of autistic traits, empathy and the quality of social relationships support this view (Knickmeyer et al., 2005, 2006; Chapman et al., 2006; Auyeung et al., 2009, 2012). Also recent neurophysiological data suggested that fetal testosterone can act as an organizing mechanism for the development of sexual dimorphisms in the brain by showing that increased fetal testosterone levels predisposes the differentiating brain to a hypermasculine profile (Lombardo et al., 2012). In relation to the present findings, it can be hypothesized that alterations (elevations/reductions) in (prenatal) sex hormonal levels are related to the observed shifts towards typical male connectivity patterns in females with ASD and shifts towards typical female connectivity patterns in males with ASD. Several reports of elevated levels of androgens and androgen-related conditions in females with ASD (Geier and Geier, 2007; Ingudomnukul et al., 2007; Bejerot et al., 2012) and recent findings of reductions in male-specific body characteristics in males with ASD (Bejerot et al., 2012) are supportive of this view.

From an evolutionary perspective, several male physical parameters determined by the action of androgens have been suggested to signal ‘hormonal health’ to potential female mates (such as nose width, jaw shape, height, body configuration and voice pitch) (Bejerot et al., 2012). It is likely that aside physical parameters, also neuronal circuits regulating social functioning
for promoting mating behavior are under strong influence of sex steroids. Disturbances in sex steroid levels may therefore cause shifts in brain development and cause alterations from the typical or ‘optimal’ brain profile. Considering that these ‘optimal’ brain profiles are most likely sex-specific, it can be conceptualized that a feminization of the ‘optimal male brain state’ in males, and a masculinization of the ‘optimal female brain state’, can give rise to an overall similar presentation of impairments in social functioning, as seen in ASD.

To date, a number of studies have explored sex-related differences in brain function, but a complex picture of sexual differentiation in the typical population seems to exist. In our study, sexual differentiation was mostly reflected by patterns of lower connectivity in typical females compared to typical males, for example for connectivity of the PCC-seed with regions in the inferior frontal gyrus and superior/middle temporal gyrus, or for the left STS-seed with the middle cingulated gyrus. Several studies similarly reported lower connectivity in females compared to males in the default mode network (Allen et al., 2011; Filippi et al., 2013), the visual network (Biswal et al., 2010; Filippi et al., 2013) and the attention network (Allen et al., 2011; Bluhm et al., 2008), but differences were not always found (Weisemann-Fogel et al., 2010; Allen et al., 2011; Tomasi and Volkow, 2012) and also a number of studies showed higher connectivity in females compared to males in these networks (e.g. for the attention network: Allen et al., 2011; Filippi et al., 2013; or for the default mode network: Bluhm et al., 2008; Biswal et al., 2010; Tomasi and Volkow, 2012; Filippi et al., 2013). Furthermore, males and females have been shown to display differential patterns of changing connectivity with age across the lifespan, suggesting for example that DMN connectivity decreases at a higher rate with age in males compared to females (Scheinost et al., 2015). To sum up, it seems that further work is needed to understand how differences in male and female ‘brain profiles’ relate to behavioral manifestations and whether and how they may contribute to heightening or lowering susceptibility for the development of neurological or psychiatric conditions.

Aside the exploration of neural masculinization mechanisms as predicted by the EMB theory, the current study additionally explored whether alterations in functional connectivity are potentially more pronounced or severe in females compared to males as predicted by the female protective factor (FPF) model (Werling and Geschwind, 2013). Particularly, in the light of reports of increased genetic loads in females with ASD compared to affected males (Sebat et al., 2007; Levy et al., 2011; Sanders et al., 2011), it has been proposed that a female-specific factor may protect females from reaching the threshold for ASD diagnosis, and that those females who are diagnosed are likely to carry a more severe ASD etiological load than affected males. In the present study however, both our behavioral and neural data do not support the view that females with ASD were more severely affected than males with ASD. At the behavioral level, our male and female cohorts—matched on age and IQ—displayed no significant sex-differences on core autistic symptom severity as measured by the ADI-R and ADOS. Females with ASD even showed tentatively lower ADOS symptom severity scores compared to males. Also at the neural level, our data provided strong indications of differential expressions of ASD in males and females rather than more pronounced or severe alterations in females compared to males with ASD. Indeed, rather than spatially extending the brain alterations seen in males, connectivity alterations in females were found in brain areas other than those affected in males. Similar to our study, Lai et al. (2013) worked with a cohort of normal- to high-functioning males and females with autism and found also no evidence of more severe female neuro-anatomical abnormalities (Lai et al., 2013). As proposed in the latter study, a potential explanation for the absence of ‘more severe’ female expressions may be that general cognitive functioning can affect how autism manifests in males and females (Lai et al., 2013). Future research is therefore needed to establish whether the absence of ‘more severe’ female expressions also holds for populations with more severe intellectual disabilities, as the possibility of an IQ-dependent female-specific protective or compensatory mechanism cannot be ruled out. A recent study showed that reductions in IQ are related to greater social impairment and reduced adaptive behavior in females with ASD (Frazier et al., 2014). Further, the well-described relationship between IQ and sex ratio in ASD prevalence has also been linked to a female-specific protective factor that is IQ-dependent (indicating IQ sex ratios of 6–8:1 among normal-functioning cohorts, and 1.7:1 among cohorts with moderate-to-severe intellectual disabilities) (Fombonne, 1999; Fombonne, 2005). The male and female cohorts used in the current study may only reflect a ‘high-functioning’ subgroup within the ASD population, meeting the stringent fMRI recruitment criteria. It is therefore unknown whether the present results will generalize to more disabled populations.

Our intrinsic functional connectivity results showed consistent patterns of hypo-connectivity in males with ASD, and hyper-connectivity in females with ASD. Overall, this observation corresponds to the broader—often male-biased—ASD neuroimaging literature, where reports of hypo-connectivity are quantitatively more abundant (Müller et al., 2011; Kana et al., 2014; Di Martino et al., 2014). Using an unbiased whole-brain voxel-based measure of functional connectivity, a recent study by Cheng et al. (2015) mainly reported decreases in functional connectivity in a large sample of ASD-subjects of the ABIDE repository. Although males and females were combined in this study, the number of female subjects was considerably smaller (85 controls, 51 ASD) compared to the number of male subjects (424 controls, 367 ASD). Particularly, at the whole brain-level, this study identified a key system in the MTG/STS region which showed reduced functional connectivity with other cortical areas such as the left superior frontal gyrus, ventromedial prefrontal cortex, the precuneus and cuneus; as well as a system in the precuneus/superior parietal lobule region which showed reduced connectivity with cuneus, MTG, superior frontal gyrus (SFG) and ventromedial prefrontal cortex (Cheng et al., 2015). Although direct comparisons between studies are difficult, our seed-to-voxel analyses of the STS and PCC regions similarly revealed reduced functional connectivity in males with ASD, particularly with frontal regions in the SFG and middle frontal gyrus. Note however that based on our diagnosis-by-sex interaction analyses, we showed that this pattern was reversed in females with ASD which highlights the importance of including sex-specific profiles in building a more differentiated model of intrinsic network abnormalities in ASD. Of note, from our study, a particular consistency emerged in terms of diagnostic-related effects in males and females for connectivity with the SFG region. Our whole-brain ROI-to-ROI analyses (both primary (Figure 4) and secondary (Supplementary Figure S4) as well as our seed-to-voxel analyses (of the left STS- and PCC-seeds (Figures 2–3C) showed opposite diagnosis effects for connections with this region, indicating hypo-connectivity in males/hyper-connectivity in females. Also relationships with symptom severity (as assessed with the SRS) were most pronounced for the SFG region, indicating that in males with ASD, decreased connectivity of the STG was predictive of higher
symptom severity, whereas in females with ASD, increased connectivity of the STG was indicative of increased symptom severity (Supplementary Figure S3A). Although preliminary, these findings may provide first indications for a potentially important role of the SFG region in defining sex-differential expressions of ASD.

Our investigation of sex-differential expressions of ASD was based on multi-site resting-state fMRI datasets of the ABIDE repository. Given the rarity of girls with ASD, coordinated efforts such as ABIDE are highly valuable for addressing understudied topics such as the exploration of neural sex differences in ASD. Reliance on multi-center datasets may however induce additional sources of variance related to site-specific differences in participant characterization and selection (e.g. age) or scan procedures and instructions. Indeed, related to the multi-site nature of the ABIDE dataset, the age range of our sample was relatively broad (7–30 years). In order to account for this variance, we included ‘age’ as a nuisance regressor in all the reported analyses, and supplementary analyses were conducted to verify whether effects were modulated by age (Supplementary Figure S5). Nevertheless, considering recent reports on age-dependent changes in functional connectivity in ASD (Alaerts et al., 2015), future research with larger male and female samples may be warranted to firmly address sex-specific expressions of ASD at different developmental stages. Further, also phenotypic characterization of the included patients was limited such that ADOS and SRS scores on symptom severity were only available for part of the included participants. Although preliminary brain-behavior correlation analyses provided indications that the observed patterns of hyper-connectivity in ASD females/hypo-connectivity in ASD males were related to increased symptom severity, one should note that these relationships were only mild and did not survive corrections for multiple comparisons. More research with more extensive participant characterization in terms of symptom severity as well as biological data (such as sex hormonal levels) would therefore be of great interest to further elucidate the mechanisms by which sex-specific neural expressions modulate the presentation of the ASD phenotype.

In summary, males and females showed a differential neural expression of ASD, characterized by predominant patterns of hypo-connectivity in males with ASD, and hyper-connectivity in females with ASD. Overall, patterns of hyper-connectivity in females with ASD reflected a shift towards the level of connectivity seen in typical males (neural masculinization), whereas patterns of hypo-connectivity observed in males with ASD reflected a shift towards the typical feminine connectivity pattern (neural feminization). These data provide novel support to the notion that ASD may constitute a disorder of sexual differentiation and androgeny rather than a disorder characterized by masculinization in both genders.

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Supplementary data

Supplementary data are available at SCAN online.

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