Towards robust and replicable sex differences in the intrinsic brain function of autism

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Abstract

Background: Marked sex differences in autism prevalence accentuate the need to understand the role of biological sex-related factors in autism. Efforts to unravel sex differences in the brain organization of autism have, however, been challenged by the limited availability of female data. **Methods**: We addressed this gap by using the largest sample of males and females with autism and neurotypical (NT) control individuals, to date (ABIDE; Autism: 362 males, 82 females; NT: 409 males, 166 females; 7-18 years). Discovery analyses examined main effects of diagnosis, sex and their interaction across five resting-state fMRI (R-fMRI) metrics (voxel-level $Z > 3.1$, cluster-level $p < 0.01$ gaussian random field corrected). Secondary analyses assessed the robustness of the results to different pre-processing approaches and their replicability in two independent samples: the EU-AIMS Longitudinal European Autism Project (LEAP) and the Gender Explorations of Neurogenetics and Development to Advance Autism Research (GENDAAR). **Results**: Discovery analyses in ABIDE revealed significant main effects across the intrinsic functional connectivity (iFC) of the posterior cingulate cortex, regional homogeneity and voxel-mirrored homotopic connectivity (VMHC) in several cortical regions, largely converging in the default network midline. Sex-by-diagnosis interactions were confined to the dorsolateral occipital cortex, with reduced VMHC in females with autism. All findings were robust to different pre-processing steps. Replicability in independent samples varied by R-fMRI measures and effects with the targeted sex-by-diagnosis interaction being replicated in the larger of the two replication samples – EU-AIMS LEAP. **Limitations**: Given the lack of *a priori* harmonization among the discovery and replication datasets available to date, sample-related variation remained and may have affected replicability. **Conclusions**: Atypical cross-hemispheric interactions are neurobiologically relevant to autism. They likely result from the combination of sex-dependent and sex-independent factors with a differential effect across cortical functional

networks. Systematic assessments of the factors contributing to replicability are needed and necessitate coordinated large-scale data collection across studies.

Background

Autism spectrum disorder (autism) is characterized by a marked male preponderance in prevalence with three times more males being diagnosed than females [1]. This pronounced sex-differential prevalence implies that sex-related biological factors are likely implicated in the neurobiology of autism. However, little is known about the differential underlying neural expressions in males and females with autism. Such knowledge could widen our understanding of potential underlying mechanisms of autism and related neurodevelopmental conditions [2].

This has motivated research into the impact of biological sex on brain organization in autism [2–5]. With the widely accepted view that the neurobiology of autism involves differences in large-scale brain networks [6,7], resting-state functional magnetic resonance imaging (R-fMRI) has proven to be a valuable complementary tool for investigating atypicalities in intrinsic functional connectivity (iFC). While the exact nature of the intrinsic brain organization in autism remains to be established [6], research on the impact of biological sex differences in autism is just beginning to emerge.

Several R-fMRI studies have focused on autism-related sex differences in iFC [2,8–17]. They vary on the extent of the functional networks and intrinsic properties examined. Most of them examined the strength of iFC between one or more regions/networks selected *a priori* [8–11,13,14], or via data-driven analyses [17]. A few others investigated either local or homotopic iFC across the whole brain [2,12,16]. Across these different efforts, the pattern of findings have also been mixed; some studies supported the predictions from the ‘extreme male brain theory’ [11,12] (i.e., shifts towards maleness in both sexes [18]), others supported

those from the ‘gender-incoherence’ theory [8,10,13,14,17] (i.e., androgynous patterns in the sexes [19]), and one other supported the coexistence of both, at least in males [2]. Specifically, both Ypma et al. [11] and Kozhemiako et al. [12,16] observed results consistent with a shift towards maleness in autism. In contrast, other studies [8,10,13], reported findings consistent with ‘gender-incoherence’ where the results largely revealed hyper-connectivity in females with autism similar to NT males and hypo-connectivity in males with autism similar to NT females. Such seeming inconsistencies in the pattern of sex-related differences were in part addressed by Floris et al. [2] who showed that, at least in males with autism, distinct patterns of atypical sex-differentiation coexist, and vary as a function of the neural networks involved. However, the intrinsic brain organization in females with autism has remained largely unclear. Scarce availability of female datasets in most studies may have contributed to this variability in findings [20,21].

Here, to examine sex-related atypicalities in both males and females in autism relative to NT controls, we used, as discovery sample, a large autism R-fMRI sample from the Autism Brain Imaging Data Sharing Exchange (ABIDE) [21,22] of both males and females of autism and controls. By aggregating neuroimaging datasets from multiple sources, this data sharing initiative has begun to provide a means to address the challenge of underrepresentation of female datasets in autism research. Examining both sexes in both autism and controls allows to directly capture not only sex differences that are common across individuals (i.e., regardless of their diagnosis [main effect of sex]), but also those that are specific to autism and point towards atypical autism-specific sex differential patterns (i.e., sex-by-diagnosis interaction effects) [4]. Unlike most prior work that focused on specific networks or circuits selected *a priori*, we investigated autism-specific sex differences in the whole-brain across multiple R-fMRI metrics in the same study. Specifically, we selected R-fMRI metrics

capturing unique aspects of the intrinsic brain organization [23], during typical development [24]. Most germane to this study, their selection was motivated by the consistent involvement in typical sex differences and to be affected by autism. They comprised: 1) posterior cingulate cortex (PCC)-iFC – e.g., [2,11,22,25–31]; 2) voxel-mirrored homotopic connectivity (VMHC) [32] – e.g., [12,31–34]; 3) regional homogeneity (ReHo) [35] – e.g., [16,31,36,37]; 4) network degree centrality (DC) [38] – e.g., [31,38–40]; and 5) fractional amplitude of low frequency fluctuations (fALFF) [22,41] – e.g., [22,31,42].

Beside the role of small female samples, prior inconsistencies in autism-related sex differences in R-fMRI can be due to other factors that can impact reproducibility. For example, while there are growing concerns on the role of pre-processing strategies [43,44], a recent study showed that their impact on autism-related mean group-differences is minimal [45]. Additionally, while several studies have reported some degree of consistency on R-fMRI findings across either independent, or partially overlapping, samples [40,46–48], results from others have raised concerns on the replicability of group-mean diagnostic effects [45,49]. However, none of these studies have explicitly examined robustness and replicability of sex-by-diagnosis interaction effects, which account for a potentially relevant source of variability in autism – biological sex. Thus, we conducted secondary analyses to assess the extent to which the pattern of findings obtained in our discovery analyses were also observed *a)* after applying different nuisance pre-processing steps that have been previously validated, though used inconsistently in the autism literature [45], and *b)* across two independent, multisite R-fMRI datasets: the EU-AIMS Longitudinal European Autism Project (LEAP) [50,51] and the Gender Explorations of Neurogenetics and Development to Advance Autism Research (GENDAAR) dataset [52] – i.e., *robustness* and *replicability*.

Methods

Discovery sample: ABIDE I and II

For discovery analyses, we examined the R-fMRI dataset with the largest number of females and males in both the autism and the NT groups available to date, selected from the Autism Brain Imaging Data Exchange (ABIDE) repositories ABIDE I and II [21,22]. For specific selection criteria, see Additional file 2 and Additional file 4. The final ABIDE I and II dataset of N=1,019 included N=82 females with autism, N=362 males with autism, N=166 neurotypical females (NT F), and N=409 neurotypical males (NT M), aggregated across 13 sites. Details on the sample selection process and criteria are provided in Supplementary methods (Additional file 2) and Additional file 4. Briefly, we selected cases between 7 -18 years of age (the ages most represented across ABIDE sites), with MRI data successfully completing brain image co-registration and transformation to standard space, with FIQ between 70-148 and with mean framewise displacement (mFD) [53] within three times the interquartile range (IQR) + the third quartile (Q3) of the sample (i.e., 0.39 mFD). Further steps included matching for mean age *across* groups and for mFD and IQ *within* diagnostic groups. This latter step limited the number of exclusions while keeping average group motion low (mFD<.2mm) and bypassing sampling biases resulting from matching neurodevelopmental conditions to NT around intrinsic features such as IQ [54,55]. At each step, any sites with less than three individual datasets per diagnostic/sex groups were excluded. Demographics and characteristics of this sample are summarized in Additional file 1: Table 1 and Additional file 2.

Discovery analysis pre-processing pipeline

We examined five whole-brain R-fMRI metrics previously reported to reflect typical sex differences and found to be atypical in autism, including 1) PCC-iFC, 2) VMHC, 3) ReHo, 4) DC and 5) fALFF (see Additional file 2).

R-fMRI image pre-processing steps included: slice time correction, 24 motion parameters regression [56], component-based noise reduction (CompCor) [57], removal of linear and quadratic trends, and band-pass filtering (0.01-0.1 Hz, for all metrics but fALFF). Functional-to-anatomical co-registration was achieved by Boundary Based Registration (BBR) using FSL FLIRT [58]. Linear and nonlinear spatial normalization of functional echo planar images (EPIs) to Montreal Neurological Institute 152 (MNI152) stereotactic space ($2mm^3$ isotropic) was done using ANTS registration (Advanced Neuroimaging Tools) [59]. Computation of voxel-mirrored homotopic connectivity (VMHC) followed registration to a symmetric template. All R-fMRI derivatives were smoothed by a 6mm FWHM Gaussian kernel. To account for site and collection time variability across each of the data collections in ABIDE I and II data repositories, site effects were removed using the ComBat function available in python [60] (https://github.com/brentp/combat.py). This approach has been shown to effectively account for scanner-related variance in multi-site R-fMRI data [60]. For further details see Additional file 2.

Group-level analyses

Statistical Z-maps were generated within study-specific functional volume masks including all voxels in MNI space present across all subjects. Sex differences were explored by fitting a general linear model (GLM) including diagnosis or sex as the regressors of interest, and age and mean framewise displacement (mFD) [53] as nuisance covariates. We did not include FIQ as a covariate as this is not optimal when comparing groups selected from populations

carrying intrinsic IQ differences such as autism and NT [55]. We assessed the main effects of diagnosis and sex, along with their interaction effects. We applied gaussian random field theory correction based on strict voxel-level threshold of $Z > 3.1$ as recommended by [61] and cluster level, $P < 0.01$, given the assessment of five R-fMRI metrics in the same study (i.e., P 0.05/5 R-MRI metrics=0.01).

Functional relevance of sex differences in autism

Post-hoc analyses were conducted to functionally characterize the sex-by-diagnosis interaction result(s). First, to explore the cognitive domains implicated in the cluster(s), we quantified the percentage of its overlap with 12 cognitive ontology maps [62] thresholded at $P = 1e{-}5$. We labelled these components based on the top five tasks each component recruits [2]. Second, we used the Neurosynth Image Decoder (http://neurosynth.org/decode/) [63] to visualize the terms most strongly associated with the significant cluster. After excluding anatomical and redundant terms (synonyms, plurals or noun/adjective/adverb equivalents), we visualized the top 27 terms showing correlations with the cluster map between $r = 0.64$ and $r = 0.10$. Third, to explore potential clinical relevance of the significant cluster, we explored brain-behavior relationships as a function of sex within the autism group. Specifically, we ran a GLM examining the interaction between biological sex and available ADOS calibrated severity total score (CSS) [64], as well as social-affect (SA) and restricted, repetitive behavior (RRB) subscores (see Additional file 2) with the dependent variable(s) being the R-fMRI metric(s) extracted from the cluster mask(s) showing a statistically significant sex-by-diagnosis effect(s).

Robustness and Replicability

Robustness. We assessed whether patterns of results from the discovery analyses were observable with two other nuisance regression pipelines that include commonly used preprocessing steps. One pipeline was global signal regression (GSR) [65] which has often been used in autism studies, and the other was Independent Component Analysis - Automatic Removal of Motion Artifacts (ICA-AROMA) [66] which is a relatively novel but increasingly utilized approach [45]. Given the scope of the present study, unlike prior work focusing on a wide range of individual preprocessing pipelines [45], we selected GSR and ICA-AROMA as examples of previously validated approaches thought to have impact on motion and physiological noise [44]. We conducted group-level analyses on the R-fMRI metrics means extracted from the masks corresponding to the clusters showing significant effects in discovery analyses and regressed out the effect of inter-individual differences in age, and mFD across all effects tested; for main diagnostic and sex comparisons, sex or diagnosis, respectively, were also regressed out.

Replicability. Similarly, we assessed whether the group patterns observed in significant clusters identified in discovery analyses, were observed in two relatively large-scale, independent datasets selected from *a)* the EU-AIMS Longitudinal European Autism Project (LEAP), a large multi-site European initiative aimed at identifying biomarkers in autism [50,51] and *b)* the Gender Explorations of Neurogenetics and Development to Advance Autism Research (GENDAAR) dataset collected by the GENDAAR consortium and shared in the National Database for Autism Research [52]. For details on autism and NT inclusion and exclusion criteria for these samples, as well as our selection process, see Additional file 2 [51,52]. The resulting EU-AIMS LEAP (N=309) R-fMRI datasets comprised N=133 males and N=43 females with autism as well as N=85 NT males, and N=48 NT females (see Additional file 3: Table S1); resulting GENDAAR (N=196) R-fMRI datasets comprised

N=43 males and N=44 females with autism, as well as N=56 NT males and N=53 NT females (see Additional file 3: Table S2). For a comparison of demographic and clinical information between ABIDE, EU-AIMS LEAP and GENDAAR, see Additional file 3: Table S3, S4 and S5. After applying the same ComBat and pre-processing pipeline used in the ABIDE-based discovery analyses to adjust for inter-site variability within EU-AIMS LEAP and GENDAAR, we extracted each of the R-fMRI metrics means from the Z-maps in masks corresponding to the clusters showing statistically significant effects in discovery analyses. We then computed group-mean comparisons and their interactions of the residuals data resulting from regressing out the same nuisance variables used in primary analyses (i.e., mFD and age across all tests, and sex or diagnosis for diagnostic or sex comparisons, respectively).

For these exploratory analyses, discovery findings were determined to be robust and/or replicable (R+) based on two criteria: *1)* the group mean difference(s) observed were in the same direction as those identified in the findings from discovery analyses [67] and *2)* their effects were not negligible as defined by Cohen' *d* [68] (i.e., any effects, small, medium or large as indexed by Cohen's $d \geq |0.2|$) which is also consistent with prior work [40].

Results

Discovery analyses – ABIDE

Main effect of diagnosis. Analyses revealed clusters showing a significant effect of diagnosis (voxel-level $Z > 3.1$; cluster-level $P < 0.01$, corrected) for three of the five R-fMRI metrics: PCC-iFC, VMHC and ReHo. These were mainly evident in anterior and posterior regions of the default network (DN) across at least two or all three R-fMRI metrics (Figure 1,

Additional file 5, and Additional file 3: Table S6). Autism-related hypo-connectivity was present for: a) PCC-iFC, VMHC and ReHo within bilateral paracingulate cortex and frontal pole, b) VMHC and ReHo in the bilateral PCC and precuneus, and c) ReHo in right insula and central operculum (Figure 1, Additional file 5 and Additional file 3: Table S6). Autism-related hyper-connectivity was only evident for PCC-iFC with left superior lateral occipital cortex, temporal occipital fusiform cortex and occipital fusiform gyrus (Additional file 5). To verify that these findings were not driven by particular acquisition site(s), post-hoc analyses computed group means for diagnostic subgroups for the R-fMRI metrics extracted at the cluster-level masks excluding one out of the 13 ABIDE sites at a time. The pattern of results was essentially unchanged (Additional file 7: Figure S2a)6.

Main effect of sex. Analyses revealed clusters showing statistically significant main sex differences (voxel-level $Z > 3.1$; cluster-level $P < 0.01$, corrected) for three metrics out of five: PCC-iFC, VMHC and ReHo. Findings involved lateral and medial portions of the DN with bilateral PCC and precuneus showing the highest overlap (see Figure 1 and Additional file 5). Specifically, regardless of diagnosis, relative to females, males showed decreased PCC-iFC with paracingulate cortex and frontal pole, right middle frontal gyrus, bilateral superior lateral occipital cortex and bilateral PCC and precuneus. Males also showed decreased VMHC and ReHo localized in PCC and precuneus. Decreased ReHo was also evident in the left angular gyrus and lateral occipital cortex in females relative to males (Figure 1, Additional file 5, and Additional file 3: Table S6). Post-hoc analyses assessing the consistency of these findings across sites, as described above, revealed a similar pattern of results (Additional file 7: Figure S2b).

Sex-by-diagnosis interaction. Statistically corrected voxel-wise analyses (voxel-level $Z > 3.1$; cluster-level $P < 0.01$) revealed a cluster of significant sex-by-diagnosis interaction only for VMHC which was localized in the dorsolateral occipital cortex (Figure 2a and Additional file 3: Table S6). Post-hoc cluster-level group means showed that NT females had higher VMHC than the three other groups, whereas autism females had lower VMHC than the three other groups (Figure 2a). Similar to the main effects, analyses assessing the consistency of these findings across sites, as described above, showed a similar pattern of results (Additional file 8).

Functional relevance of autism-related sex differences

Post-hoc analyses to functionally characterize this VMHC sex-by-diagnosis interaction indicated that the VMHC cluster in superior lateral occipital cortex overlapped with cognitive maps involved in higher-order visual, oculomotor, cognitive flexibility and language-related processes (Figure 3a). Further, as shown in Figure 3b, the most common terms were primarily related to lower-order visual processing and higher-order visual cognition, such as 'visuospatial' and 'spatial attention.' To explore potential clinical relevance of the VMHC dorsolateral occipital cluster, we explored brain-behavior relationships as a function of sex, within the autism group using three available ADOS scores (calibrated severity total score, and non-calibrated social affect and RRB subscores; see Supplemental Material). Although not surviving a strict Bonferroni correction for multiple testing (i.e., 0.05/3=0.02), an interaction effect was observed for ADOS social affect scores. It revealed that more severe social deficits ($F_{(1,311)}$=4.44, p=0.036) were associated with decreased VMHC in females with autism (r=-0.29), but not in males with autism (r=0.03). Given that ABIDE data were aggregated and released when calibrated social affect scores [69] were not available to assess potential differences in language abilities and age, analyses were repeated after including

ADOS module (ADOS Module 2 to 4) as a nuisance covariate: results remained unchanged ($F_{(1,306)}$=5.0, p=0.026) as they did also after removing the few data with the less represented ADOS module 2 (see Additional file 2: Supplementary methods). There were no significant findings, with regard to the CSS total score and non-calibrated RRB sub-score (Figure 3c), even at an exploratory statistical threshold of P<0.05.

Robustness

The same pattern of results identified in primary analyses was observed in the results pre-processed using GSR or ICA-AROMA, across the three R-fMRI metrics in all the clusters identified in primary analyses across effects (Cohen's *d* range=|0.2-0.6|; Figure 2b, Figure 4, Additional file 6, Additional file 3: Table S6).

Replicability

Main effects of diagnosis. Main effects of diagnosis showed higher replicability (i.e., non-negligible Cohen's *d* effects showing a similar group differences pattern as observed in discovery analyses) in GENDAAR than in EU-AIMS LEAP. Specifically, across the three R-fMRI metrics that were significant in discovery analyses, four of the seven clusters (57%; Cohen'*d* range=|0.2-0.4|) in GENDAAR, while only one (14%; Cohens' *d*=|0.2|) in EU-AIMS LEAP, were replicated. Nevertheless, this one cluster showing decreased ReHo in ASD vs. NT in insula and central operculum was replicated across all samples (Figure 4, Additional file 9: Figure S3a, and Additional file 3: Table S6).

Main effects of sex. Across all three R-fMRI metrics, the main effects of sex observed in primary analyses was evident in both independent samples, for most clusters in the EU-AIMS LEAP (90%; 9/10; Cohen' *d* range=|0.2-0.5|) and GENDAAR (60%; 6/10; Cohen' *d*

range=|0.2-0.3|); (Figure 4, Additional file 9: Figure S3b and Additional file 3: Table S6). Notably, the pattern of typical sex differences localized along the default network midline (i.e., decreased VMHC, ReHo and PCC-iFC) was replicated across both independent samples (Figure 6b).

Sex-by-diagnosis interaction effect. The pattern of autism-related VMHC sex differences observed in discovery analyses in the superior lateral occipital cortex was clearly observed in the EU-AIMS LEAP dataset (Cohen's *d*=0.34); (Figure 2c; Figure 4, Additional file 3: Table S6). In the GENDAAR dataset, while group means in males with autism, NT males and NT females showed a similar direction as in the ABIDE discovery findings, females with autism differed in magnitude and in the direction of group differences, resulting in a negligible effect with Cohen's *d*=0.09 (Figure 2c).

Discussion

We examined autism-related sex differences for intrinsic functional brain organization across multiple R-fMRI metrics in a large discovery sample of males and females with autism relative to age-group matched NT selected from the ABIDE repositories [21,22]. Analyses revealed significant main effects of sex and diagnosis across intrinsic functional connectivity (iFC) of the posterior cingulate cortex, regional homogeneity and voxel-mirrored homotopic connectivity (VMHC) in several cortical regions. Notably, effects converged along the midline of the default network. In contrast, sex-by-diagnosis interactions were limited to VMHC in the superior lateral occipital cortex. This result, placed in the context of sex and diagnostic main effects on interhemispheric homotopic connectivity in cortical regions, suggests that atypical interhemispheric interactions are pervasive in autism but reflect a combination of sex-independent (i.e., main effect of diagnosis common across both sexes)

and sex-dependent (i.e., sex-by-diagnosis interaction) effects, each specific to different functional cortical system. Sex-by-diagnosis interaction was robust to distinct pre-processing strategies as those observed for main effects. Further, despite the lack of *a priori* harmonization for data acquisition among the three samples, it was replicable in the larger of the two independent samples (i.e., EU-AIMS LEAP). These findings together with largely replicable main effects of sex with variable replicability of main diagnostic effects by sample, suggest on the one hand that inter-sample replicability of R-fMRI can be feasible in autism when sources of variability in diagnostic groups are accounted for in samples sized properly to account for such variability. On the other hand, they highlight the urgent need to obtain multiple harmonized datasets properly powered to systematically address and understand sources of heterogeneity, including and beyond the role of biological sex.

Sex-dependent and independent atypical interhemispheric interactions in autism

VMHC reflects inter-hemispheric homotopic relations. Its strength has been suggested to index coordinated cross-hemispheric processing: *stronger* VMHC index weaker hemispheric specialization and vice versa [32,70]. Several lines of evidence support the notion that the neurobiology of autism is related to atypical hemispheric interactions, including homotopic connectivity and hemispheric lateralization [34,71–80]. VMHC and functional hemispheric lateralization have also been shown to be sex-differential in NT [32,81,82]. The dorsolateral occipital association cortex identified in our discovery analyses is known to serve hemispherically specialized processes, such as visuospatial coordination [83]. Thus, our findings of NT males' VMHC in dorsolateral lateral occipital cortex being lower than NT females are consistent with the notion of increased hemispheric lateralization in this cortical region in NT males relative to NT females. In our data, females with autism instead showed even lower VMHC than NT males, while males with autism showed slightly higher VMHC

than NT males. This pattern is indicative of ‘gender-incoherence’ [19] as males and females with autism display the opposite pattern expected in NT per their biological sex. Findings of ‘gender incoherence’ have been reported in earlier neuroimaging studies of autism using different modalities [11–13,84–86]. Among them, several R-fMRI studies explicitly focusing on detecting diagnosis-by-sex interactions (i.e., the regression model included a sex-by-diagnosis interaction term) yielded a pattern of results consistent with ‘gender incoherence’. In contrast, other studies [11–13] reported a pattern consistent with the ‘extreme male brain theory’ [18] – i.e., a shift towards maleness in both females and males with autism. While the seemingly diverging conclusions of these two sets of studies may be attributed to methodological differences, such as the extent of brain networks explored and the statistical modelling employed, findings from our prior work suggest that both shifts towards either maleness or femaleness co-occur in the intrinsic brain of males with autism, in a network-specific manner [2]. However, such prior work did not include female data, thus by not directly assessing sex-by-diagnosis interactions, unlike the present study, results could not point to patterns affecting diagnostic differences between the sexes vs. those common to autism across both sexes [4]. This is relevant for efforts focusing on identifying underlying mechanisms of autism. Findings resulting from sex-by-diagnosis interactions may shed light on sex differential mechanisms that go awry in autism and may reflect sex-specific susceptibility mechanisms. On the other hand, diagnostic atypicalities common for both sexes may reflect factors central to the emergence of autism, regardless of whether they overlap with patterns known to be differential between sexes [87]. Interestingly, a recent study based on a sample selected from GENDAAR [15] revealed that the iFC between the nucleus accumbens (selected *a priori*) and in a region of the dorsolateral occipital cortex partially overlapping with that identified by our VMHC analyses, was differentially modulated by the aggregate number of oxytocin receptor risk alleles in females with autism vs. NT females and

vs. males with autism. Although VMHC was not directly tested in that earlier study [15], its result in dorsolateral occipital cortex is consistent with our observation of atypical sexual differentiation of this region of the visual network and suggest the need for future whole-brain studies of oxytocin effects in autism.

Along with the sex-dependent autism patterns, our analyses found statistically significant main effects of diagnosis in inter-hemispheric interactions indexed by VMHC. These were localized along the midline of the DN (paracingulate/frontal cortex consistently and PCC/precuneus) where main effects of PCC-iFC and ReHo also converged. Our results are consistent with prior reports of atypical intrinsic organization of the DN in autism [11,22,25,88–90]. Together they support the role for a common, sex-independent DN atypicality in autism. This is also supported by a recent autism neurosubtyping study that identified three latent iFC factors, all sharing DN atypicalities along with their neurosubtype-specific patterns [91]. Building on this evidence to disentangle the specific roles of each of the factors affecting autism in sex-independent and sex-dependent ways, a necessary next step is to engage in novel large-scale data collection efforts including more female data.

Robustness, replicability and sources of variability

The growing awareness of the replication crisis in neuroscience [92–94] motivated our analyses examining robustness and replicability of findings. While a comprehensive and systematic reproducibility assessment is beyond the scope of the study, here we focused on examining whether the findings observed in the discovery analyses were also seen after using different preprocessing pipelines – *robustness* – as well as in fully independent, albeit convenience (i.e., not harmonized *a priori* with each other), samples – *replicability*. To this extent, given the lack of consensus on quantitative metrics of replicability and to avoid

chasing after p values, we opted to consider findings to be robust and/or replicable for any non-negligible effects per Cohen's *d* [68], including small, but not negligible effects (i.e., Cohen's > *d* =0.2). Furthermore, given their distributed and heterogenous nature [6], it is plausible that atypicalities in the autism connectome stem from a combination of differently sized effects, as shown for autism in other biological domains such as genetics [95,96].

With this in mind, across the two preprocessing methods examined here, the patterns of findings were consistent with those observed in discovery analyses across all R-fMRI metrics and effects. These robustness results are consistent with a prior study by He and colleagues [45] reporting that differences in a wider range of pre-processing pipelines have marginal effects on variation in diagnostic group average comparisons. Our study confirms and builds on this earlier report by extending findings of robustness to sex group mean differences and their interactions with diagnosis.

A more nuanced picture emerged from the inter-sample analyses as replicability varied by sample, across the effects and R-fMRI metrics examined. Specifically, while inter-sample main effects of sex were moderately to largely replicable across R-fMRI metrics on both independent samples (~60 to 90% of the clusters in GENDAAR and EU-AIMS-LEAP, respectively), replicability of diagnostic effects significantly varied by sample (57 to 14%) across R-FMRI metrics. This is at least in part consistent with findings by King et al. [49] that, depending on the R-fMRI feature examined, diagnostic group differences varied across samples. Even in this scenario, King et al. also reported that findings of decreased homotopic connectivity in autism were relatively more stable than other R-fMRI metrics [49]. This combined with replicability of our VMHC sex-by-diagnosis interaction findings in the larger of the two independent samples (EU-AIMS LEAP), suggests that measures of homotopic

connectivity may have a relevant biological relevance for autism. It is also possible that given its moderate to high test-retest reliability, VMHC is more suitable in efforts assessing replicability [97,98].

The striking clinical and biological heterogeneity in autism should be considered as a major contributor to discrepancies in findings of studies focusing on the main effects of diagnostic group means contrasts/interactions [99–101]. We interpret our replicability findings on diagnostic effects and, in turn, diagnosis-by-sex interactions against this background. Inter-sample differences may have contributed to the more variable results of replicability on the diagnosis main effects. These include IQ, autism symptom level, and age. For example, the EU-AIMS LEAP sample was on-average older, had lower VIQ and most notably, lower symptom severity across all subscales of the ADOS and ADI-R than the ABIDE sample. On the other hand, the GENDAAR sample (which has greater number of replicable diagnostic mean group patterns) did not differ from ABIDE in these variables, except for mean age. Furthermore, a fact that is often neglected, is that the NT groups may also present with considerable sample heterogeneity between studies [99,102]. For instance, our NT controls in the EU-AIMS LEAP sample had lower VIQ than both ABIDE and GENDAAR NT controls. This has potentially influenced the low replicability of diagnosis main effects in EU-AIMS LEAP.

In contrast, sex-by-diagnosis effect on VMHC in the dorsolateral occipital cortex was replicable in the larger samples, the EU-AIMS LEAP, but not in GENDAAR. Small samples introduce larger epistemic variability (i.e., greater variation related to known and unknown confounds) [103]. Increasing the number of subjects/data allows mitigating epistemic variability and, thus, capturing the underlying variability of interest. Thus, although the rate

of EU-AIMS LEAP replicability for the main effect of diagnosis was limited, accounting for biological sex, a known key source variability in autism, may have contributed to a replicable sex-by-diagnosis pattern in this larger sample. In line with sample size concerns, using four datasets sized between 36 and 44 individuals selected from the ABIDE repository, He et al. [45] found low similarity rates of diagnostic group-level differences on the strength of iFC edges in contrast with the largely similar pattern of results across pipelines. Of note, unlike prior efforts [45,49], we controlled for site effects within each of the samples (i.e., ABIDE, GENDAAR and EU-AIMS LEAP), using ComBat. These results are consistent with earlier reports of reproducible imaging biomarkers even when accounting for inter-site differences in multisite datasets such as the ABIDE I repository [46]. Future large-scale harmonized data collections are needed to control and assess the impact of inter-sample variability. Taken together, these findings highlight that sample differences can impact replicability.

Beyond clinical and biological sources of variation, samples may differ in MRI acquisition methods, as well as in approaches used to mitigate head motion during data collection and its impact on findings [104]. Adequately controlling for head motion remains a key challenge for future studies assessing inter-sample replicability. For the present study, we excluded individuals with high motion, retained relatively large samples with group average low motion (mean $\pm$ standard deviation range mFD range=0.09-0.16 $\pm$ 0.06-0.10 mm) as well as included mFD at the second-level analyses as a nuisance covariate. Overall, the extent to which each sample-related factors affect replicability needs to be systematically examined in future well-powered studies. Only this type of studies will allow for emerging subtyping approaches to dissect heterogeneity by brain imaging features using a range of data-driven methods [105], including normative modelling [106–108].

Inter-sample differences and methodological differences, beyond nuisance regression, may have contributed to some differences in findings between the present and earlier studies, conducted with independent or partially overlapping samples [10,22,40]. For example, Alaerts et al. [10] also examined sex-by-diagnosis interaction in PCC iFC in a dataset selected from ABIDE I only. Although the pattern of results was consistent with the 'gender incoherence' model, the resulting clusters were distinct from those identified in the present study. Along with differences in samples selected from the same data repositories, other studies differed with ours in the inclusion of sex-by-diagnosis interaction [16], the extent of the whole-brain voxel-based analyses [14,15], or the statistical threshold utilized [22]. Nevertheless, it is remarkable that even in light of these differences, consistent results have emerged including the role of the 'gender incoherence' model for females with autism, atypical inter-hemispheric interactions in autism, and sex-dependent and sex-independent atypical intrinsic brain function across distinct functional networks.

Limitations

Along with the inter-sample differences resulting from the lack of sufficiently available harmonized inter-site replication datasets in the field, other limitations of this study should be addressed in future efforts. One regards the lack of measures differentiating the effects of sex vs. that of gender so as to disentangle their relative roles (e.g., gender-identity and gender-expression) in the intrinsic brain properties [109]. Further, in-depth cognitive measures to directly characterize the role of VMHC findings were not available. Additional behavioral measures are needed to establish whether our result in VMHC of the dorsolateral occipital cortex mainly applies to low-level (bottom-up) visual processing differences or higher-level (top-down) attentional/controlled processes in males and females with autism. As a neurodevelopmental disorder, autism shows striking inter-individual differences in clinical

and developmental trajectories, as well as outcomes. Thus, age may influence symptom presentation [110,111] and neurobiology [12,106,112]. Despite the considerable size of the samples available for this study, it is still difficult to sufficiently cover a broad age range across both males and females and diagnostic groups across contributing sites and to evaluate age effects appropriately. Even larger cross-sectional samples are needed to derive meaningful age-related information that ultimately require confirmation in longitudinal study designs.

Conclusions

The present work revealed sex differences in the intrinsic brain of autism, particularly in dorsolateral occipital interhemispheric interactions, which were robust to pre-processing pipeline decisions and replicable in the larger of the two independent samples. While differences in nuisance regression pipelines have little influence on the consistency of findings, sample heterogeneity represents a challenge for replicability of findings. Lateralized cognitive functions and cross-hemispheric interactions should be further explored in relation to sex differences in autism while addressing this challenge with future harmonized data acquisition efforts with even larger samples.

List of abbreviations

ABIDE: Autism Brain Imaging Data Exchange

ASD: Autism spectrum disorder

DC: Degree centrality

DN: Default network

EPI: Echo-planar image

LEAP: Longitudinal European Autism Project

fALFF: Fractional amplitude of low frequency fluctuations

FIQ: Full-scale IQ

GENDAAR: Gender Explorations of Neurogenetics and Development to Advance Autism Research

GSR: Global Signal Regression

ICA-AROMA: Independent Component Analysis - Automatic Removal of Motion Artifacts

iFC: Intrinsic functional connectivity

mFD: Mean framewise displacement

NDAR: National Database for Autism Research

NT: Neurotypical

PCC: Posterior cingulate cortex

ReHo: Regional Homogeneity

R-fMRI: Resting-state functional magnetic resonance imaging

VMHC: Voxel-mirrored homotopic connectivity

Ethics approval and consent to participate

ABIDE I and II: All contributions were based on studies approved by the local Institutional Review Boards, and data were fully anonymized (removing all 18 HIPAA (Health Insurance Portability and Accountability)-protected health information identifiers, and face information from structural images).

EU-AIMS LEAP: Ethical approval was obtained through ethics committees at each site.

GENDAAR: Informed assent and consent were obtained from all participants and their legal guardians, and the experimental protocol was approved by the Institutional Review Board at each participating site.

Consent for publication

Not applicable

Availability of data and materials

ABIDE I and II: Data are freely accessible in the publicly available Autism Brain Imaging Data Exchange repository (http://fcon_1000.projects.nitrc.org/indi/abide).

EU-AIMS LEAP: Data are currently only available for sites involved in data collection, but data (starting with the first wave) will be made available on request.

GENDAAR: Anonymized data are publicly available through the National Database for Autism Research (NDAR) and access can be requested via https://nda.nih.gov/about.html.

Competing interests

ADM receives royalties from the publication of the Italian version of the Social Responsiveness Scale—Child Version by Organization Speciali, Italy. JKB has been a consultant to, advisory board member of, and a speaker for Takeda/Shire, Medice, Roche, and Servier. He is not an employee of any of these companies and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, or royalties. CFB is director and shareholder in SBGneuro Ltd. TC has received consultancy from Roche and Servier and received book royalties from Guildford Press and Sage. DM has been a consultant to, and advisory board member, for Roche and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. TB served in an advisory or consultancy role for Lundbeck, Medice, Neurim Pharmaceuticals, Oberberg GmbH, Shire, and Infectopharm. He received conference support or speaker's fee by Lilly, Medice, and Shire. He received royalties from Hogrefe, Kohlhammer, CIP Medien, Oxford University Press; the present work is unrelated to these

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Authors' contributions (in alphabetical order by contribution)

ADM and DLF have contributed to the study' s conception; ADM, DLF, JOAF, M-CL, MPM have contributed to distinct aspects of the study design and interpretation of all findings; DLF and JOAF conducted the analyses; SG has provided support in all data analyses requiring CPAC; ADM, DLF, JOAF have generated figures and tables; ADM and DLF drafted the manuscript; ADM, DLF, JOAF, M-CL, and MPM have revised and edited multiple versions of the manuscript; CFB, MM, MO, have organized the EU-AIMS LEAP data and edited latest manuscript versions and its revisions; CFB, CE, CM, DGMM, EL, FDA, GD, JKB, JT, SB-C, TC, TB, SD have contributed to the coordination, data acquisition and coordination of the EU-AIMS LEAP project, as well as edited later versions of the manuscript. All authors read and approved the manuscript.

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Figure Captions

Fig 1. Overlap across R-fMRI metrics for main effects of diagnosis and sex

Upper panel: the surface inflated maps depict the extent of overlap across any of three resting state fMRI (R-fMRI) metrics for clusters showing significant main effects of diagnosis (left) and sex (right). Purple clusters represent areas of significant group differences emerging for only one of any of the three R-fMRI measures, orange and yellow clusters indicate measures with overlap among 2 and 3 R-fMRI measures (see Additional file 5 for statistical maps of main effects for each R-fMRI metric). Cluster masks are overlaid on inflated brain maps generated by BrainNet Viewer. Lower panel: for each of the yellow and orange clusters in panel A, the table lists the cluster's anatomical area label based on the Harvard Oxford atlas, the specific R-fMRI metrics involved, and the group difference direction (ASD<NT or M<F in blue, ASD>NT or M>F in red; see Additional file 3: Table S6 for more details). **Abbreviations:** L= Left hemisphere; R= Right hemisphere, PCG/FP=Paracingulate cortex/frontal pole, ACC=Anterior cingulate cortex, PCC/Prec=Posterior cingulate cortex/precuneus, ASD=autism spectrum disorder, NT=neurotypical, M=Males, F=Females).

Fig 2. Sex-by-diagnosis interaction effect, its robustness and replicability

a) On the right, the surface maps show the cluster with a significant ($Z > 3.1$, $P<0.01$) sex-by-diagnosis interaction for voxel-mirrored homotopic connectivity (VMHC) resulting from discovery analyses in the ABIDE sample using the component-based noise reduction (CompCor) pipeline. The statistical Z maps are overlaid on inflated brain maps generated by BrainNet Viewer. **b)** The upper panels show the pattern of VMHC group means in males and females by each diagnostic group (ASD and NT) extracted from the same cluster in pre-processed data following two alternative denoising pipelines, Global Signal Regression

(GSR, left) and Independent Component Analysis – Automatic Removal of Motion Artifacts (ICA-AROMA, right). Results show a pattern similar to the primary results observed in discovery analyses with moderate to high effect sizes (Cohen's *d* range= 0.5-0.6). **2c)** The lower graph shows replicability in two independent samples: the Gender Explorations of Neurogenetics and Development to Advance Autism Research (GENDAAR) and the EU-AIMS Longitudinal European Autism Project (LEAP). The pattern of results was replicable in the EU-AIMS LEAP (N=309) with a moderate effect size (Cohen's *d* = 0.34) and had a negligible effect size in GENDAAR (N=196; Cohen's *d* = 0.09). For all graphs VMHC data are shown as residuals obtained after regressing out mean framewise displacement and age effects. **Abbreviations:** L=left, R=right, A=anterior, P=posterior.

Fig 3. Functional relevance of sex-by-diagnosis interaction in VMHC

a) The radar plot shows the percentage (0-80%) of overlap between the voxels in the dorsolateral occipital cluster showing a significant VMHC sex-by-diagnosis interaction in discovery analyses and the 12 Yeo cognitive ontology probability maps [62] (probability threshold at *P* = 1e-5) for cognitive components C1-C12. As in Floris et al. [2], we labelled each component based on the top five tasks reported to be most likely recruited by a given component. **b)** Word cloud based on the top 27 terms showing correlations between *r*=0.64 to *r*=0.10 associated with the same VMHC cluster based on the Neurosynth Image Decoder. **c)** Sex-differential association between each individual's VMHC at the cluster showing a significant sex-by-diagnosis interaction in primary analyses and available ADOS social-affect uncalibrated sub-scores in males and females with ASD. VMHC data are shown as residuals obtained after regressing out mean framewise displacement and age effects. While males showed no significant associations at corrected and uncorrected thresholds, females with

lower dorsolateral occipital VMHC showed more severe social-affect symptoms at uncorrected statistical threshold ($F_{(1,311)}$=4.44, p=0.036).

Fig 4. Robustness and replicability summary

a) The histogram summarizes the percentage of clusters showing a robust and replicable pattern of results as that observed in discovery analyses in the ABIDE sample for main effects of diagnosis (Dx; green; N=10 clusters), sex (yellow; N=7 clusters) and their interaction (blue; N=1 cluster) across three R-fMRI metrics. All findings were robust to different preprocessing pipelines. Across R-fMRI metrics, main sex effects were moderately (60%) to largely (90%) replicable across independent samples: Gender Explorations of Neurogenetics and Development to Advance Autism Research (GENDAAR) and the EU-AIMS Longitudinal European Autism Project (LEAP), respectively. Replicability for main effects of diagnosis was moderately replicable in GENDAAR (57%) and minimally replicable in EU-AIMS LEAP (14%). The VMHC pattern observed for sex-by-diagnosis interaction in primary analyses was replicated in EU-AIMS LEAP only. **b)** Surface conjunction maps show the clusters replicated in GENDAAR only (G, purple), EU-AIMS LEAP only (E, blue) and in both samples (G&E, red) for each effect separately. Cluster masks are overlaid on inflated brain maps generated by BrainNet Viewer.

Additional files

Additional file 1

Title: Table 1

File format: docx

Description: Characterization of sample merged across ABIDE I and II

Additional file 2

Title: Supplemental Material

File format: docx

Description: Supplemental Methods

Additional file 3

Title: Supplementary Tables

File format: docx

Description: Characterization of EU-AIMS LEAP and GENDAAR samples, comparison between samples and summary table of main effects.

Additional file 4

Title: Selection flowchart for the ABIDE sample

File format: tif

Description: The flowchart illustrates the selection process resulting in the final ABIDE I and II combined sample of 1019 subjects; each step is detailed in Supplementary material.

Abbreviations: ASD=autism spectrum disorder, NT=neurotypical, A I=ABIDE I.

Additional file 5

Title: Main effects of diagnosis and sex in the ABIDE discovery sample

File format: png

Description: Significant results ($Z > 3.1$, $P < 0.01$, corrected) of voxel-wise discovery analyses conducted in the ABIDE dataset for main effects (ME) of diagnosis (left) and sex (right) for seed-based intrinsic functional connectivity of the posterior cingulate cortex-(PCC), voxel mirror homotopic connectivity (VMHC), and Regional Homogeneity (ReHo). Significant clusters are overlaid on inflated brain maps generated by BrainNet Viewer. No significant effects were detected for degree centrality or fractional amplitude of low

frequency fluctuations. **ME Diagnosis**: PCC-iFC: bilateral paracingulate cortex and frontal pole (PCG/FP), superior lateral occipital cortex (sLOC), temporal occipital fusiform cortex and occipital fusiform gyrus (TOFC/OFC); VMHC: bilateral posterior cingulate gyrus and precuneus (PCC/Prec), PCG/FP; ReHo: PCG/FP, central operculum and insula (CO/Ins). **ME Sex**: PCC-iFC: bilateral sLOC, middle frontal gyrus (MFG), bilateral PCC/Prec, bilateral PCG/FP; VMHC: bilateral PCC/Prec, bilateral anterior cingulate cortex (ACC); ReHo: bilateral PCC, angular gyrus and lateral occipital cortex (AnG/LOC). See Additional file 3: Table S6 for details on each cluster sizes. *Due to processing failure of two subjects for VMHC, the sample size comprised 1017 subjects instead of 1019.

Additional file 6

Title: Robustness to nuisance corrections of main effects of diagnosis and sex

File format: png

Description: Cluster-level replication of the results emerging from the voxel-wise discovery analyses in the ABIDE dataset preprocessed using CompCor for the statistically significant main effects of diagnosis **(Figure S1a)** and sex (**Figure S1b)** after preprocessing with GSR and with ICA-AROMA. The second column on the left shows the clusters ($Z > 3.1$, $P < 0.01$, corrected) with significant diagnostic and sex effects for posterior cingulate cortex intrinsic functional connectivity (PCC-iFC), voxel-mirrored homotopic connectivity (VMHC), and regional homogeneity (ReHo). Results are overlaid on inflated brain maps generated by BrainNet Viewer. The bar plots represent the residual means resulting from regressing out diagnosis or sex effects depending on the desired main effect from the cluster means. **Figure S1a)** The ABIDE GSR and ABIDE ICA-AROMA columns illustrate, for each of these R-fMRI indices, the diagnostic group mean pattern across clusters with a diagnostic effect size of $d \geq 0.2$. Color codes: Red=ASD; Green=NT. PCC-iFC: bilateral paracingulate cortex and frontal pole (PCG/FP), superior lateral occipital cortex (sLOC), temporal occipital fusiform

cortex and occipital fusiform gyrus (TOFC/OFC); VMHC: bilateral posterior cingulate gyrus and precuneus (PCC/Prec), PCG/FP; ReHo: PCG/FP, central operculum and insula (CO/Ins). ABIDE GSR: 7 out of 7 main effects of diagnosis replicated (100%). ABIDE ICA-AROMA: 7 out of 7 main effects of diagnosis replicated (100%) **Figure S1b)** The ABIDE GSR and ABIDE ICA-AROMA columns illustrate, for each of these R-fMRI indices, the sex group mean pattern across clusters with an effect size of Cohen' $d \geq 0.2$. Color codes: Blue=males; Pink=females. PCC-iFC: bilateral sLOC, middle frontal gyrus (MFG), bilateral PCC/Prec, bilateral PCG/FP; VMHC: bilateral PCC/Prec, bilateral anterior cingulate cortex (ACC); ReHo: bilateral PCC, angular gyrus and lateral occipital cortex (AnG/LOC). ABIDE GSR: 10 out of 10 main effects of diagnosis replicated (100%). ABIDE ICA-AROMA: 10 out of 10 main effects of diagnosis replicated (100%). Due to processing failure of two subjects for VMHC, the sample size comprised 1017 subjects instead of 1019. VMHC data are shown as residuals obtained after regressing out mean framewise displacement and age effects. **Abbreviations:** ASD=autism spectrum disorder, NT=neurotypical, M=males, F=females, CompCor=component base noise reduction, GSR=Global Signal Regression, ICA-AROMA=independent component analysis – automatic removal of motion artifacts, PCC-iFC=posterior cingulate cortex intrinsic functional connectivity (x=0, y=-53, z=26), VMHC=voxel-mirrored homotopic connectivity, ReHo=regional homogeneity, L=left, R=right, R+=replication based on same direction of results and Cohen's $d \geq 0.2$, R-=non-replication of results (displayed in gray plots).

Additional file 7

Title: Stability of main effects

File format: png

Description: Inter-site stability was assessed after extracting group means at masks corresponding to the clusters showing significant main effects of diagnosis (**Figure S2a**) and

sex (**Figure S2b**) in the discovery analyses and then deriving the group mean when leaving one acquisition site out at the time. The pattern of results was unchanged. Different ABIDE sites are color-coded on legend on the side. Due to processing failure of two subjects for VMHC, the sample size comprised 1017 subjects. **Abbreviations**: ASD=autism spectrum disorder, NT=neurotypical, PCC-iFC=posterior cingulate cortex intrinsic functional connectivity (x=0, y=-53, z=26), VMHC=voxel-mirrored homotopic connectivity, ReHo=regional homogeneity, L=left, R=right. Different sites in ABIDE are color-coded on the top left. Data are shown as residuals obtained after regressing out mean framewise displacement and age effects.

Additional file 8

Title: Stability of sex-by-diagnosis interaction effect

File format: tif

Description: Inter-site stability of the sex-by-diagnosis interaction pattern was assessed after extracting group means at the mask corresponding to the clusters showing a significant interaction in the discovery analyses and then deriving the group mean when leaving one acquisition site out at the time. The pattern of results was unchanged. Different sites in ABIDE are color-coded in the legend on the right. Due to processing failure of two subjects for VMHC, the sample size comprised 1017 subjects. **Abbreviations**: ASD=autism spectrum disorder, NT=neurotypical, PCC-iFC=posterior cingulate cortex intrinsic functional connectivity (x=0, y=-53, z=26), VMHC=voxel-mirrored homotopic connectivity, ReHo=regional homogeneity, L=left, R=right. Different sites in ABIDE are color-coded on the top left.

Additional file 9

Title: Replicability of main effects of diagnosis and sex

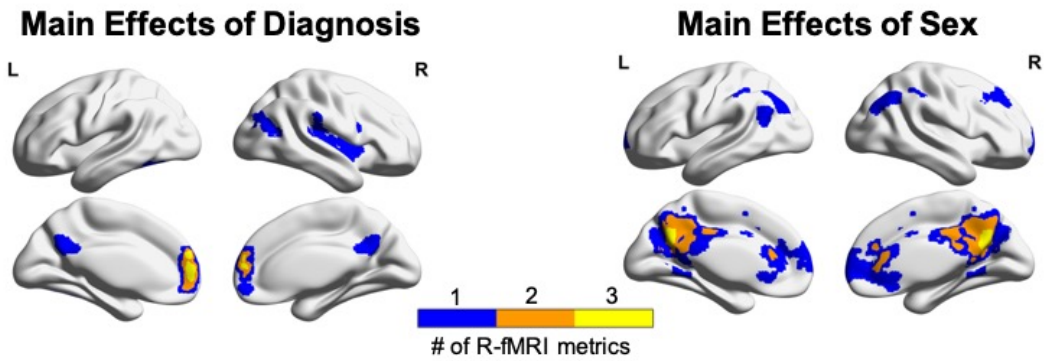
File format: png

Description: Cluster-level replication of the results emerging from the voxel-wise analyses in the ABIDE dataset for main effects of diagnosis (**Figure S3a**) and sex (**Figure S3b**) in the Gender Explorations of Neurogenetics and Development to Advance Autism Research (GENDAAR) and the EU-AIMS Longitudinal European Autism Project (LEAP) samples. The ABIDE column on the left shows the clusters ($Z > 3.1$, $P < 0.01$, corrected) with significant diagnostic and sex effects for posterior cingulate cortex intrinsic functional connectivity (PCC-iFC), voxel-mirrored homotopic connectivity (VMHC), and regional homogeneity (ReHo). No significant effects were detected for degree centrality and fractional amplitude of low frequency fluctuations. Results are overlaid on inflated brain maps generated by BrainNet Viewer. The bar plots represent the residual means resulting from the linear Gaussian regression for each group. **Figure S3a)** The GENDAAR and EU-AIMS LEAP columns illustrate, for each R-fMRI index, the diagnostic group mean pattern across clusters with a diagnostic effect size of $d \geq 0.2$. Color codes: Red=ASD; Green=NT. PCC-iFC: bilateral paracingulate cortex and frontal pole (PCG/FP), superior lateral occipital cortex (sLOC), temporal occipital fusiform cortex and occipital fusiform gyrus (TOFC/OFC); VMHC: bilateral posterior cingulate gyrus and precuneus (PCC/Prec), PCG/FP; ReHo: PCG/FP, central operculum and insula (CO/Ins). GENDAAR: 4 out of 7 main effects of diagnosis replicated (57.1%); EU-AIMS LEAP: 1 out of 7 main effects of diagnosis replicated (14.3%). **Figure S3b)** The GENDAAR and EU-AIMS LEAP columns illustrate, for each of these R-fMRI index, the sex group mean pattern across clusters with an effect size of $d \geq 0.2$. Color codes: Blue=males, Pink=females. PCC-iFC: bilateral sLOC, middle frontal gyrus (MFG), bilateral PCC/Prec, bilateral PCG/FP; VMHC: bilateral PCC/Prec, bilateral anterior cingulate cortex (ACC); ReHo: bilateral PCC, angular gyrus and lateral occipital cortex (AnG/LOC). GENDAAR: 5 out of 10 main effects of sex replicated (50%); EU-AIMS

LEAP: 9 out of 10 main effects of sex replicated (90%). *Due to processing failure of two subjects for VMHC, the sample size comprised 1017 subjects instead of 1019.

Abbreviations: ASD=autism spectrum disorder, NT=neurotypical, M=males, F=females, PCC-iFC=posterior cingulate cortex intrinsic functional connectivity (x=0, y=-53, z=26), VMHC=voxel-mirrored homotopic connectivity, ReHo=regional homogeneity, L=left, R=right, R+=replication based on same direction of results and Cohen's $d \geq 0.2$, R-=non-replication of results.

Figure 1



Main effect	Region with overlap	R-fMRI metric		
		PCC-iFC	VMHC	ReHo
DX	Bilateral PCG/FP			
Sex	Bilateral ACC			
	Bilateral PCC/Prec			

ASD<NT
M<F

ASD>NT
M>F

Figure 2

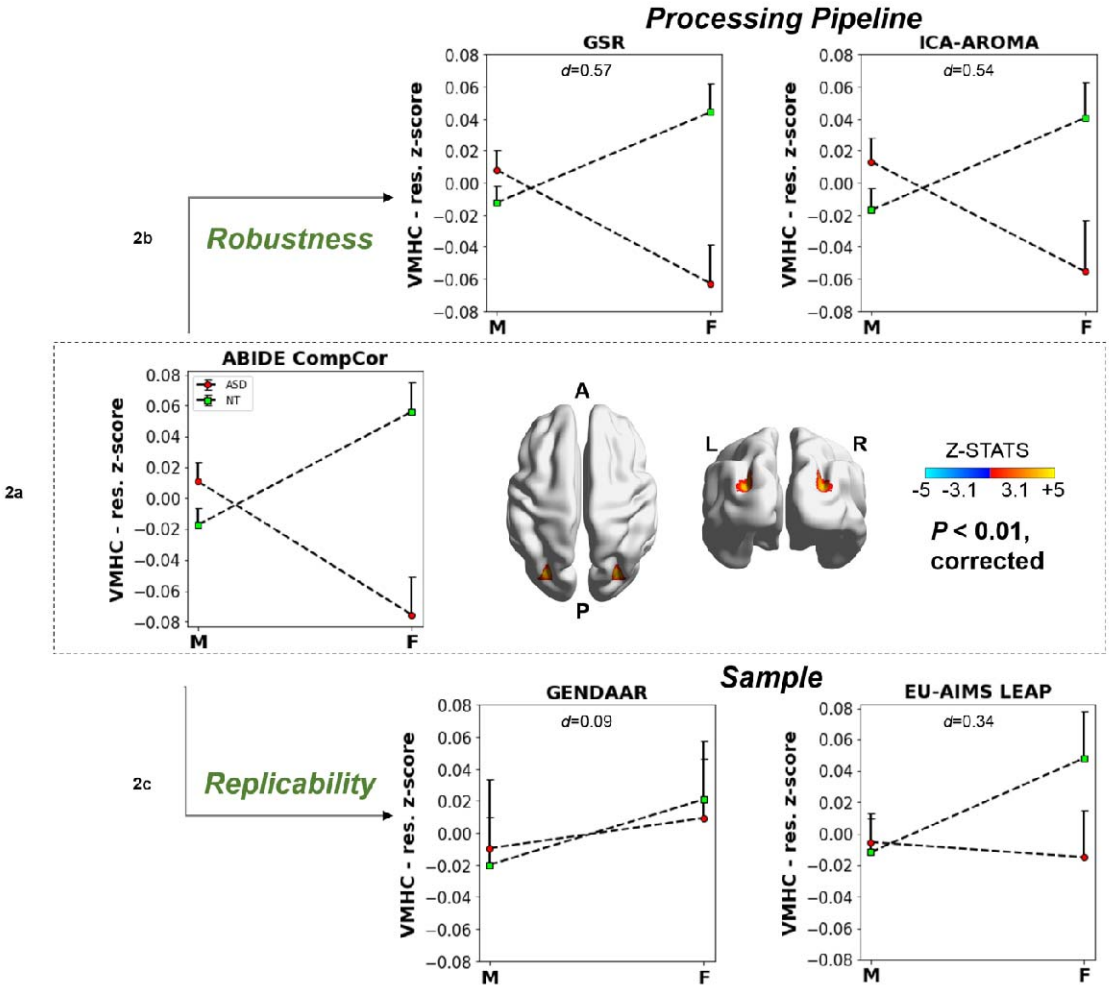

Processing Pipeline
GSR
d=0.57
ICA-AROMA
d=0.54
2b
Robustness
VMHC - res. z-score
M
F
ABIDE CompCor
ASD
NT
2a
A
P
L
R
Z-STATS
-5 -3.1 3.1 +5
P < 0.01,
corrected
Sample
GENDAAR
d=0.09
EU-AIMS LEAP
d=0.34
2c
Replicability

Figure 3

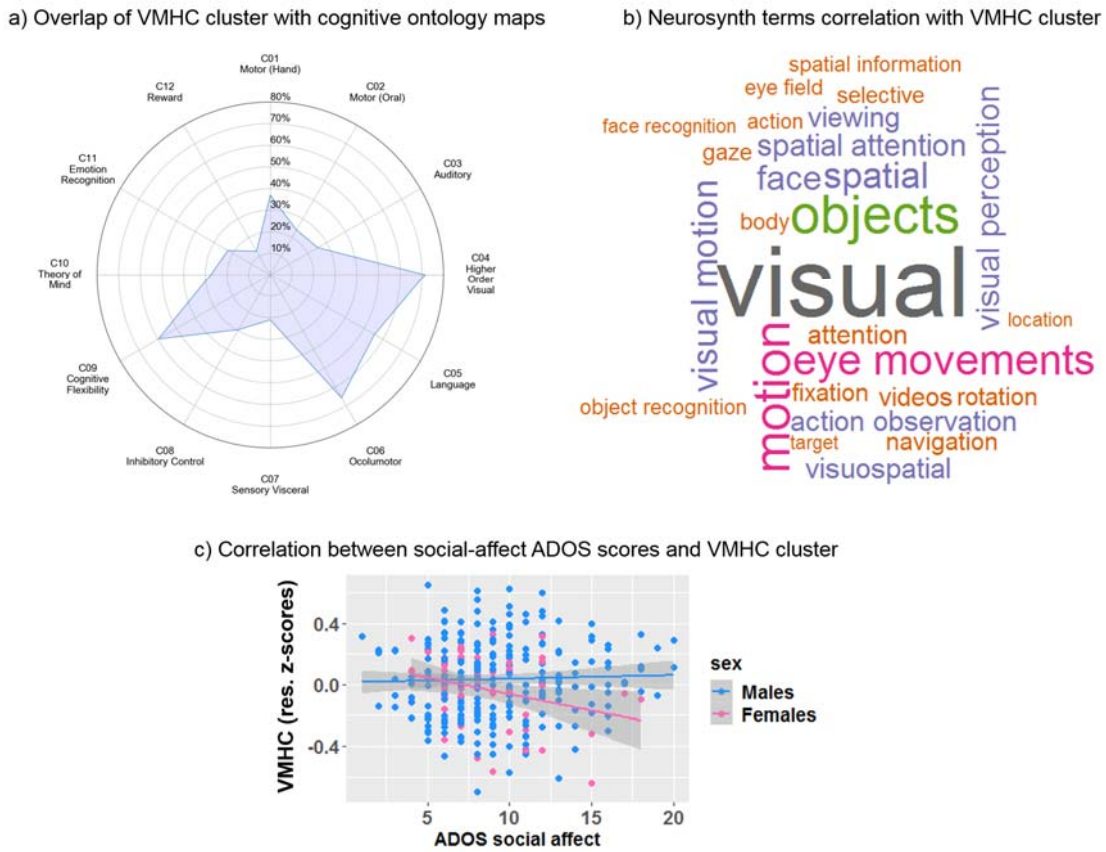
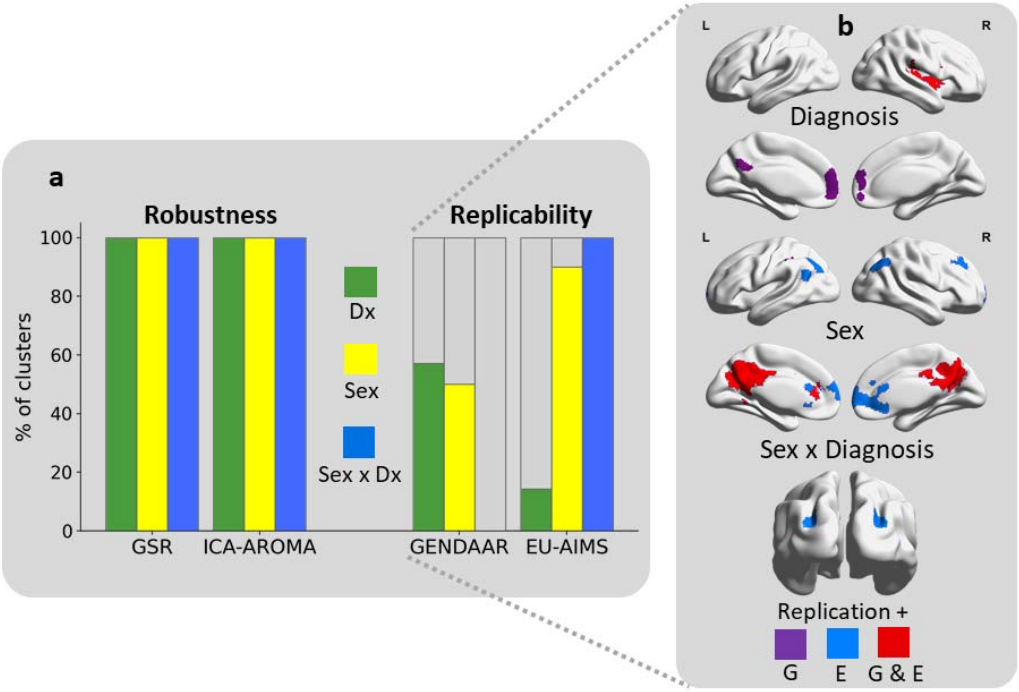

a) Overlap of VMHC cluster with cognitive ontology maps
C01 Motor (Hand)
C02 Motor (Oral)
C03 Auditory
C04 Higher Order Visual
C05 Language
C06 Ocolumotor
C07 Sensory Visceral
C08 Inhibitory Control
C09 Cognitive Flexibility
C10 Theory of Mind
C11 Emotion Recognition
C12 Reward
80%
70%
60%
50%
40%
30%
20%
10%
b) Neurosynth terms correlation with VMHC cluster
spatial information
eye field
selective
face recognition
action
viewing
gaze
spatial attention
face
spatial
body
objects
visual motion
visual
visual perception
location
attention
motion
eye movements
object recognition
fixation
videos
rotation
action observation
target
navigation
visuospatial
c) Correlation between social-affect ADOS scores and VMHC cluster
VMHC (res. z-scores)
0.4
0.0
-0.4
5
10
15
20
ADOS social affect
sex
Males
Females

Figure 4


a
Robustness
Replicability
% of clusters
100
80
60
40
20
0
GSR
ICA-AROMA
GENDAAR
EU-AIMS
Dx
Sex
Sex x Dx
b
L
R
Diagnosis
Sex
Sex x Diagnosis
Replication +
G
E
G & E