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 male and female autistic and neurotypical (NT) control individuals, to date (ABIDE; Autism: 362 males, 82 females; NT: 410 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. Secondary analyses assessed the robustness of primary 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 and subcortical regions. Sex-by-diagnosis interactions were confined to VMHC in dorsolateral occipital cortex with reduced VMHC in females with autism. Findings were highly robust to different pre-processing steps and replicable in another sample. Specifically, the sex-by-diagnosis interaction replicated in the larger of the two replication samples - EU-AIMS LEAP. **Conclusions**: Results emphasize that atypical cross-hemispheric interactions are neurobiologically relevant to autism. Systematic assessments of the factors contributing to their replicability are needed and necessitate coordinated large-scale data collection across studies.

Introduction

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 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 etiological mechanisms of autism and related neurodevelopmental conditions (2).

Recently, 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 biological sex differences in autism is even more in its infancy.

To date, only a handful of R-fMRI studies have focused on autism-related sex differences in iFC (8–11). They vary on methodological aspects such as the extent of network approach utilized and intrinsic properties examined. Findings are also mixed; some support the predictions from the 'extreme male brain theory' (12) (i.e., shifts towards maleness in both sexes), others support the "gender-incoherence" theory (13) (i.e., androgynous patterns in the sexes). Specifically, both Ypma et al. (2016) (10) and Kozhemiako et al. (2019) (11) observed a similar pattern of results consistent with a shift towards maleness model of

autism. In contrast, both Alaerts et al. (2016) (9) and Smith et al. (2019) (8) 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. (2018) (2) who showed that in males with autism, atypicalities in sex-differential iFC coexist, and vary as a function of the neural networks involved. However, the iFC pattern in females with autism remains unclear. Scarce availability of female datasets, particularly in neuroimaging (ranging between N=16 to N=55 in the above-mentioned studies), may have contributed to this variability in findings (14,15).

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) (15,16) of both males and females. 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. Unlike most prior work that focused on specific networks or circuits selected *a priori*, we investigated autism-specific sex differences in the whole-brain. We selected R-fMRI metrics capturing unique aspects of brain organization (17), known to be both affected by autism (2,16,18) and differ across the sexes typically (19–22). They comprised: 1) posterior cingulate cortex (PCC)-iFC, 2) voxel-mirrored homotopic connectivity (VMHC) (23), 3) regional homogeneity (ReHo) (24), 4) network degree centrality (DC) (25), and 5) fractional amplitude of low frequency fluctuations (fALFF) (26).

Last, 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 impact of pre-processing strategies (27,28), this has recently been shown to have little impact on autism-related group-differences (29). Additionally, while some studies have reported a certain degree of consistency of findings on R-fMRI data across samples (30–33), results from others have raised concerns (29,34). However, none of these studies have explicitly examined sex-by-diagnosis interaction effects. Thus, we conducted secondary analyses to assess *a)* the robustness of findings obtained in our discovery sample to differences in nuisance pre-processing steps that have been previously validated, though used inconsistently in autism research, and *b)* the replicability of findings in two independent, multisite, large-scale R-fMRI datasets: the EU-AIMS Longitudinal European Autism Project (LEAP) (35,36) and the Gender Explorations of Neurogenetics and Development to Advance Autism Research (GENDAAR) dataset (37).

Methods and Materials

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, selecting data from the Autism Brain Imaging Data Exchange (ABIDE) repositories ABIDE I and II (15,16). For specific selection criteria, see Supplemental Methods and Supplemental Fig 1. 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. Demographics and characteristics of this sample are summarized in Table 1 and Supplemental Material.

Discovery analysis pre-processing pipeline

We examined five R-fMRI metrics previously reported to reflect typical sex differences (19–22) and autism-related intrinsic brain properties (16,18,38,39): 1) PCC-iFC (38,40), 2) VMHC (23), 3) ReHo (41), 4) DC (25), and 5) fALFF (26) (see Supplemental Material).

R-fMRI image pre-processing steps included: slice time correction, 24 motion parameters regression (42), component-based noise reduction (CompCor) (43), 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 (44). 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) (45). 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 (46) (https://github.com/brentp/combat.py). This approach has been shown to effectively account for scanner-related variance in multi-site R-fMRI data (46). For further details see Supplemental Material.

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 and sex as the regressors of interest, and age and mean framewise displacement (mFD) (47) as nuisance covariates. We computed the main effects of diagnosis and sex, along with their two-way sex-by-diagnosis interaction.

Multiple comparisons were corrected based on Gaussian random field theory (voxel-level $Z > 3.1$; cluster level, $P < 0.05$, corrected).

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 (48) 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/) (49) 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 ADOS social-affect (SA) and restricted, repetitive behavior (RRB) subscores and calibrated severity total score (CSS) (50) (see Supplemental Methods) with the dependent variable being the R-fMRI metrics of interest across the cluster(s) showing a statistically significant effect(s).

Robustness

We assessed the robustness of results to nuisance pre-processing choices using two other nuisance regression pipelines. One – global signal regression (GSR) (51) – has often been used in autism studies; the other – Independent Component Analysis - Automatic Removal of Motion Artifacts (ICA-AROMA) (52) – as a relatively novel but increasingly utilized approach (29). We conducted group on the R-fMRI metrics means extracted from the masks

corresponding to the clusters showing significant effects in discovery analyses. Further, to assess stability of results across the 13 ABIDE acquisition sites included in this study, we computed group means for diagnostic and/or sex subgroups excluding one out of 13 sites at a time and examined consistency of the group average patterns of results.

Replicability

To assess the replicability of the significant clusters identified in our discovery sample, we selected two large-scale, independent R-fMRI datasets from *a)* the EU-AIMS Longitudinal European Autism Project (LEAP), a large multi-site European initiative aimed at identifying biomarkers in autism (35,36) 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 (37). For details on autism and NT inclusion and exclusion criteria for these samples, as well as our selection process, see Supplemental Material (36,37). The resulting EU-AIMS LEAP (N=309) R-fMRI datasets comprised N=133 males with autism, N=43 females with autism, N=85 NT males, and N=48 NT females (see Supplemental Table 1); resulting GENDAAR (N=196) datasets comprised N=43 males with autism, N=44 females with autism, N=56 NT males and N=53 NT females (see Supplemental Table 2). For a comparison of demographic and clinical information between ABIDE, EU-AIMS LEAP and GENDAAR, see Supplemental Tables 3, 4 and 5.

After applying ComBat and the pre-processing pipeline used in the ABIDE based discovery analyses, we extracted the R-fMRI metrics means from the EU-AIMS LEAP and GENDAAR Z-maps from masks corresponding to the clusters showing significant effects in the ABIDE discovery analyses; group-mean comparisons and their interactions were assessed. A finding

was determined to be replicable (R+) based on two criteria: *1)* the effect of interest for a particular contrast has Cohen's $d \geq 0.2$ (see Supplemental Material), and *2)* the group mean difference(s) was in the same direction as that identified in the discovery analyses.

Results

Discovery analyses – ABIDE

Main effect of diagnosis. Clusters showing a significant effect of diagnosis (voxel-level $Z > 3.1$; cluster level $P < 0.05$, corrected) were evident mainly in anterior and posterior regions of the default network (DN). Autism-related hypo-connectivity was present for: a) PCC-iFC, VMHC and ReHo within bilateral medial frontal gyrus, b) VMHC and ReHo in the bilateral PCC, and c) ReHo in right insula (Fig 1). Autism-related hyper-connectivity was only evident for PCC-iFC with bilateral fusiform gyrus and right superior occipital gyrus (Fig 1).

Main effect of sex. Statistically significant sex differences (voxel-level $Z > 3.1$; cluster level $P < 0.05$, corrected) involved lateral and medial portions of the DN. Specifically, regardless of diagnosis, relative to females, males showed decreased PCC-iFC with medial prefrontal cortex, right middle temporal gyrus, bilateral middle frontal, superior frontal and angular gyrus. Males also showed decreased VMHC in most of these regions and the thalamus. Decreased ReHo – a proxy of local iFC – was also evident in the left superior temporal gyrus in males relative to females, who, in contrast, showed increased ReHo in PCC (Fig 1).

Sex-by-diagnosis interaction. Voxel-wise analyses revealed a cluster of significant sex-by-diagnosis interaction for VMHC in the dorsal lateral occipital cortex (Fig 2a). 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 (Fig 2b). Post-hoc analyses to functionally characterize this VMHC sex-by-diagnosis interaction indicated that the VMHC cluster overlapped with cognitive maps involved in higher-order visual, oculomotor, cognitive flexibility and language-related processes (Fig 4a). Further, as shown in Fig 4b, 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 occipital cluster, we explored brain-behavior relationships as a function of sex, within the autism group using available ADOS scores (see Supplemental Material). We found a significant interaction showing more social deficits as assessed by the social affect ADOS score (F=4.44, p=0.03), with decreased VMHC in females with autism (r=-0.29), while there was no such relationship in males with autism (r=0.03). There were no significant findings with regard to the CSS and RRB scores.

Robustness analysis

Using GSR or ICA-AROMA, results for significant main effects of sex and diagnosis were robust across R-fMRI metrics and clusters with a Cohen's d range=0.2-0.4 and group means in the same direction as the primary discovery analyses (Supplemental Fig 2). The same was true for the sex-by-diagnosis interaction effect on VMHC (Fig 3a) with moderate to high effects sizes of Cohen's d =0.57 using GSR, and Cohen's d=0.54 using ICA-AROMA (Fig 3a). Additionally, when we left out one site at a time, the pattern of results for main effects of diagnosis (Supplemental Fig 3), sex (Supplemental Fig 4) and sex-by-diagnosis interaction (Supplemental Fig 5) remained virtually unchanged.

Replicability analyses – EU-AIMS LEAP and GENDAAR

Across all three R-fMRI metrics, the main effect of sex was largely replicated in both samples: 79% (11/14) clusters identified in discovery analyses were replicated in EU-AIMS LEAP and 36% (5/14) in GENDAAR (Supplemental Fig 6). On the other hand, main effects of diagnosis showed higher replicability in GENDAAR than EU-AIMS LEAP. Specifically, five of the nine clusters (56%) identified in discovery analyses were replicated in GENDAAR, while only 1 out of 9 (11%, PCC-iFC) was replicated in EU-AIMS LEAP (Supplemental Fig 6). Regarding the sex-by-diagnosis interaction VMHC cluster finding, in the EU-AIMS LEAP dataset, the group mean patterns replicated those observed in discovery analyses (Cohen's d=0.34). 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 Cohen's d=0.09 (Fig 3b).

Discussion

We examined autism-related sex differences for intrinsic functional brain organization in a large discovery sample of males and females with autism relative to age-group matched NT selected from the ABIDE repositories (15,16). Sex-by-diagnosis interactions were confined to homotopic connectivity (VMHC) in the dorsal lateral occipital cortex. This, along with main effects of diagnosis on VMHC in other cortical regions, suggests that atypical interhemispheric interactions are pervasive in autism and likely reflect a combination of sex-independent (main effect of diagnosis common across both sexes) and sex-dependent (sex-by diagnosis interaction) factors. This finding was highly robust to distinct pre-processing strategies and, despite the lack of *a priori* harmonization, it was replicable in the larger of two

independent samples (i.e., EU-AIMS LEAP). This suggests that replicability of R-fMRI is feasible in autism; though, it also highlights the urgent need to obtain multiple harmonized datasets properly powered to account for and understand sources of heterogeneity - including and beyond the role of biological sex.

The role of interhemispheric interactions in autism

Several lines of evidence support the notion that the neurobiology of autism is related to atypical hemispheric interactions, including homotopic connectivity and hemispheric lateralization (53–63). Interestingly, as VMHC reflects inter-hemispheric homotopic relations, its strength has been suggested to index coordinated cross-hemispheric processing with *stronger* VMHC indexing weaker hemispheric specialization (23,64) and vice versa. VMHC and functional hemispheric lateralization have been shown to be sex-differential in NT (23,65,66). The occipital association cortex identified in our discovery analyses is known to serve hemispherically specialized processes, such as visuospatial coordination (67). Thus, our findings of NT males' VMHC in 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 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" (13) 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 (10,11,68–70). Among them, two 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, two other R-fMRI studies (10,11) reported a pattern consistent with the 'extreme male brain theory' (12) – i.e., a shift towards maleness in both females and males with autism. While the seemingly diverging conclusions of these two set studies may be attributed to methodological differences such as the extent of brain networks explored and the statistical modelling employed, finding from our recent work suggest that both shifts towards either maleness or femaleness co-occur in the intrinsic brain of males with autism (38). Although prio work did not include females, its findings along with those from the present study suggest that the mechanisms underlying VMHC sex differences in autism may be more heterogeneous for males than for females and that their impact on intrinsic functional network properties vary accordingly. Additionally, consistent with prior reports of sex-differential brain-behavior relationships in autism (69,71), our results showed that females with autism with lower VMHC in the lateral occipital cortex exhibited more severe social-affective ADOS ratings, a relationship that was absent in males with autism. Taken together these findings underscore that gender-incoherent brain patterns may have differential functional implications for males and females with autism. Finally, our main effect findings of atypical autism-related VMHC in cortical area other than association occipital cortex, suggest that a combination of sex-independent and sex-dependent factors may exist and differentially affect cortical homotopic interactions in autism. Notably, the main VMHC effect of diagnosis was localized along the midline of the DN. This result, along with consistent prior reports of atypical intrinsic organization of the DN in autism (10,72–76), point towards a common sex-independent DN disruption 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 (77). To disentangle the specific roles of each of these factors affecting sex-independent and sex-dependent autism, a

necessary first step is to engage in novel large-scale data collection efforts including more female data.

Replicability and sources of variability

The growing awareness of the replication crisis in neuroscience (78–80) motivated our analyses on replicability of findings, in independent, albeit of convenience, samples. First, results showed that, while inter-sample main effects of sex were largely replicable across R-fMRI metrics, replicability of diagnostic effects varied by sample and R-fMRI metric. This is consistent with findings by King *et al.* (2019) (34) revealing that, depending on the R-fMRI feature examined, diagnostic group differences varied across ABIDE samples. Yet, King et al (34) also reported that findings of decreased homotopic connectivity in autism were relatively more stable across samples (34). This combined with replicability of our VMHC sex-by-diagnosis interaction findings, 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 (81,82).

The striking clinical and biological heterogeneity characteristic of autism should also be considered as a major contributor to discrepancies in findings of studies focusing on main group average (83–85). We interpret our replicability results against this background. We note that our significant sex-by-diagnosis effect on VMHC in the occipital cortex was replicable in the largest samples, the EU-AIMS LEAP, but not in the GENDAAR sample. Small samples may introduce larger epistemic variability (i.e., greater variation related to known and unknown confounds) (86). Increasing the number of subjects/data allows mitigating epistemic variability and, thus, capturing the underlying variability of interest. Similarly, using three datasets of N=30 individuals selected from ABIDE, He *et al.*, (2020)

(29) found low similarity rates of diagnostic group-level differences on functional connectome edges. Notably, the study by He and colleagues also reported that differences in pre-processing pipeline have marginal effects on variation in results. This finding is in line with our observation on robustness across R-fMRI metrics and stresses that sample differences have greater impact on replicability. Of note, within samples (i.e ABIDE, GENDAAR and EU-AIMS), here, we controlled for site effects using ComBat and showed a largely stable pattern of results across ABIDE sites. These results are consistent with earlier reports of reproducible imaging biomarkers even when accounting for inter-site differences in multisite datasets like the ABIDE I repository (30). Yet, future large-scale harmonized data collections are needed to control and possibly assess the impact of inter-sample variability.

In the present study, several inter-sample differences may have contributed to our more variable results of replicability on the main effect of diagnosis. These include IQ, autism severity, and age. For example, the EU-AIMS LEAP sample was on-average older, had lower VIQ and lower symptom severity across all subscales of the ADOS and ADI-R than the ABIDE sample. On the other hand, the GENDAAR sample did not differ from ABIDE in all these variables except for mean age. As a neurodevelopmental disorder, individuals with autism show striking differences in clinical and developmental trajectories, as well as outcomes. Thus, age has a crucial influence on symptom presentation (87,88) and neurobiology (11,89,90). Despite the considerable size of ABIDE, to sufficiently cover a broad age range across both males and females, even larger cross sectional samples are needed to derive meaningful age-related information that ultimately require require longitudinal study designs. Furthermore, a fact that is often neglected, is that the NT may also present with considerable sample heterogeneity between studies (83,91). For instance, here, our NT controls in the EU-AIMS LEAP sample had lower VIQ than both ABIDE and

GENDAAR NT. This has also potentially influenced the low replicability of main effects of diagnosis in EU-AIMS LEAP. Beyond clinical and biological sources of variation, samples further differed in MRI acquisition methods, as well as in approaches used to mitigate head motion and its impact on findings (94). For the present study, we excluded individuals with high motion while retaining a large sample and included mFD at the second-level analyses as a nuisance covariate. Thus, controlling adequately for head motion remains a key challenge for future studies assessing inter-sample replicability. Overall the extent to which each of these sample-related factors affect replicability needs to be systematically examined in future well-powered studies. This type of studies will also allow to apply emerging subtyping approaches to dissect heterogeneity by brain imaging features using a range of data-driven methods (92), including normative modelling (62,90,93).

Along with the inert-samples differences resulting from the lack of available harmonized replication datasets in the field, other limitations in this study exists should be addressed in future efforts. One regards the lack of measures differentiating the effects of sex versus that of gender so to disentangle their relative roles (e.g., gender-identity and gender-expression) in the intrinsic brain properties (95). Further, in depth cognitive measures to directly characterize the role of VMHC findings were not available in ABIDE. Additional behavioral measures are needed to establish whether our result mainly applies to low-level (bottom-up) visual processing differences or higher-level (top-down) attentional/controlled processes in males and females with autism.

Conclusions

The present work revealed sex differences in the intrinsic brain of autism, particularly in occipital interhemispheric interactions, which were robust to pre-processing pipeline

decisions and partially replicable. While difference in nuisance regression pipelines have little influence on the consistency of results, sample heterogeneity represents a challenge for efforts assessing consistency of findings. Thus, lateralized cognitive functions and cross-hemispheric interactions should be further explored in relation to sex differences in autism while addressing this challenge with harmonized efforts.

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Figure Captions

Fig 1. Main effects of diagnosis and sex in the ABIDE discovery sample.

Significant results ($Z > 3.1$, $P < 0.05$, corrected) of voxel-wise discovery analyses in the ABIDE dataset for main effects (ME) of diagnosis (left) and sex (right) for seed based intrinsic functional connectivity analyses of the posterior cingulate cortex- (PCC), voxel mirror homotopic connectivity (VMHC), and Regional Homogeneity (ReHo). Significant cluster are overlaid on inflated brain maps generated by BrainNet Viewer. No significant effects were detected for DC and fALFF. **ME Diagnosis**: PCC-iFC: a) right superior occipital gyrus, b) left fusiform gyrus (FFG), c) right FFG, d) bilateral medial frontal gyrus (MFG); VMHC: a) bilateral PCC, b) bilateral MFG; ReHo: a) bilateral PCC, b) bilateral MFG, c) right insula. **ME Sex**: PCC-iFC: a) bilateral MFG, b) bilateral PCC, c) right middle temporal gyrus (MTG), d) left inferior parietal lobe (IPL), e) left IPL, f) right superior frontal gyrus (SFG); VMHC: a) bilateral PCC, b) bilateral MFG, c) bilateral thalamus d) bilateral IPL, e) right MTG; ReHo: a) bilateral PCC, b) left SFG, c) left superior temporal gyrus.
*Due to processing failure of two subjects for VMHC, the sample size comprised 1017 subjects instead of 1019.

Fig 2. Sex-by-diagnosis interaction effect in the ABIDE discovery sample

2a. Voxel-wise analyses in the ABIDE discovery sample – preprocessed with the CompCor pipeline – yielded a cluster with significant ($Z > 3.1$, $P < 0.05$, corrected) sex-by-diagnosis interaction in the voxel-mirrored homotopic connectivity (VMHC) of the superior lateral occipital cortex. Results are overlaid on inflated brain maps generated by BrainNet Viewer.
2b. Group mean and standard deviations in males and females with autism spectrum disorder (ASD; red) and neurotypicals (NT; green). Females (F) with ASD showed decreased VMHC

compared to all three other groups. **Abbreviations**: CompCor=component-based noise reduction, M=males, L=left, R=right, A=anterior, P=posterior.

Fig 3. Robustness and replicability of sex-by-diagnosis interaction effects

Graphs depict group mean and standard deviations in males and females with autism spectrum disorder (ASD; red) and neurotypicals (NT; green) males (M) and females (F) in voxel-mirrored homotopic connectivity (VMHC) extracted form a mask corresponding to the cluster identified in discovery analyses in the ABIDE sample using component-based noise reduction (CompCor) pipeline having significant sex by diagnosis effect (middle left graph and centered cluster projected on inflated surface brains). **3a.** The upper panels show robustness to 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 primary results from discovery analyses with moderate to high effect sizes (Cohen's *d* range= 0.5-.6) **3b**. The lower panels show replicability in two independent samples. The pattern of results was replicable in the EU-AIMS LEAP (N=309) with a moderate effect size (Cohen's *d* range= 0.3), but had a minimal effect size in GENDAAR (N=196). **Abbreviations:** L=left, R=right, A=anterior, P=posterior.

Fig 4. Functional relevance of sex-by-diagnosis interaction in VMHC

4a) The radar plot shows the percentage (0-80%) of overlap between the voxels in the cluster showing a significant VMHC sex-by-diagnosis interaction in discovery analyses and the 12 Yeo cognitive ontology probability maps (48) (probability threshold at *P* = 1e-5) for cognitive components C1-C12. As in Floris *et al.* (2018) (2), we labelled each component based on the top five tasks reported to be most likely recruited by a given component. **4b)** 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. **4c)** Sex-differential association between VMHC and ADOS social affect sub-scores in males and females with ASD. While males show no association, females with lower VMHC in the cluster identified in discovery analyses show more severe social-affect symptoms.

Supplemental Fig 1. Selection flowchart for the ABIDE sample

The flowchart illustrates the selection process resulting in the final ABIDE I and II combined sample of N=1019 each step is detailed in Supplementary material. **Abbreviations:** ASD=autism spectrum disorder, NT=neurotypical, A I=ABIDE I.

Supplemental Fig 2. Robustness to nuisance corrections of main effects of diagnosis and sex

Cluster level replication of the results emerging from the voxel-wise discovery analyses in the ABIDE dataset preprocessed using CompCor for the main effects of diagnosis **(2a)** and sex (**2b)** after preprocessing with GSR and with ICA-AROMA. The second column on the left shows the clusters ($Z > 3.1$, $P < 0.05$, corrected) with significant diagnostic and sex effects for PCC-iFC, VMHC, and ReHo. No significant effects were detected for DC and fALFF. 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. **2a)** 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: a) right superior occipital gyrus, b) left fusiform gyrus (FFG), c) right FFG, d) bilateral medial frontal gyrus (MFG); VMHC: a) bilateral PCC, b) bilateral MFG; ReHo: a) bilateral PCC, b) bilateral MFG, c) right insula. ABIDE GSR: 9 out of 9 main effects of diagnosis replicated (100%). ABIDE ICA-AROMA:

6 out of 9 main effects of diagnosis replicated (66.7%). **2b)** 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 $d \geq 0.2$. Color codes: Blue=males; Pink=females. PCC-iFC: a) bilateral MFG, b) bilateral PCC, c) right middle temporal gyrus (MTG), d) left inferior parietal lobule (IPL), e) left IPL, f) right superior frontal gyrus (SFG); VMHC: a) bilateral PCC, b) bilateral MFG, c) bilateral thalamus d) bilateral IPL, e) right MTG; ReHo: a) bilateral PCC, b) left SFG, c) left superior temporal gyrus. ABIDE GSR: 14 out of 14 main effects of diagnosis replicated (100%). ABIDE ICA-AROMA: 14 out of 14 main effects of diagnosis replicated (100%). 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, 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).

Supplemental Fig 3. Stability of main effects of diagnosis

Inter-site stability of diagnostic group differences was assessed after extracting group means at masks corresponding to the clusters showing significant diagnostic effects in the discovery analyses and then leaving the groups mean of one acquisition site at the time. The pattern of results was virtually 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 N=1017 subjects. **Abbreviations**: 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. ASD=autism spectrum disorder, NT=neurotypical, VMHC=voxel-mirrored homotopic connectivity.

Supplemental Fig 4. Stability of main effects of sex

Inter-site stability of sex group differences were assessed after extracting group means at masks corresponding to the clusters showing a significant sex effects in the discovery analyses and then leaving the groups mean of one acquisition site at the time. The pattern of results was virtually 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 N=1017 subjects. **Abbreviations**: 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. ASD=autism spectrum disorder, NT=neurotypical, VMHC=voxel-mirrored homotopic connectivity.

Supplemental Fig 5 Stability of sex-by-diagnosis interaction

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 leaving the groups mean of one acquisition site at the time. The pattern of results was virtually 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 N=1017 subjects. **Abbreviations**: 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. ASD=autism spectrum disorder, NT=neurotypical, VMHC=voxel-mirrored homotopic connectivity.

Supplementary Figure 6 Replicability of main effects of diagnosis and sex

Cluster level replication of the results emerging from the voxel-wise analyses in the ABIDE dataset for main effects of diagnosis (**6a**) and sex (**6b**) in the GENDAAR and EU-AIMS LEAP samples. The ABIDE column on the left shows the clusters ($Z > 3.1$, $P < 0.05$, corrected) with significant diagnostic and sex effects for PCC-iFC, VMHC, and ReHo. No significant effects were detected for DC and fALFF. 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. **6a)** The GENDAAR and EU-AIMS LEAP columns illustrate, for each 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: a) right superior occipital gyrus, b) left fusiform gyrus (FFG), c) right FFG, d) bilateral medial frontal gyrus (MFG); VMHC: a) bilateral PCC, b) bilateral MFG; ReHo: a) bilateral PCC, b) bilateral MFG, c) right insula. GENDAAR: 5 out of 9 main effects of diagnosis replicated (55.6%); EU-AIMS LEAP: 1 out of 9 main effects of diagnosis replicated (11.1%). **6b)** The GENDAAR and EU-AIMS LEAP columns illustrate, for each of these R-fMRI indices, the sex group mean pattern across clusters with an effect size of $d \geq 0.2$. Color codes: Blue=males, Pink=females. PCC-iFC: a) bilateral MFG, b) bilateral PCC, c) right middle temporal gyrus (MTG), d) left inferior parietal lobule (IPL), e) left IPL, f) right superior frontal gyrus (SFG); VMHC: a) bilateral PCC, b) bilateral MFG, c) bilateral thalamus d) bilateral IPL, e) right MTG; ReHo: a) bilateral PCC, b) left SFG, c) left superior temporal gyrus. GENDAAR: 5 out of 14 main effects of sex replicated (35.7%); EU-AIMS LEAP: 11

out of 14 main effects of sex replicated (78.6%). *Due to processing failure of two subjects for VMHC, the sample size comprised 1,017 subjects instead of 1,019.

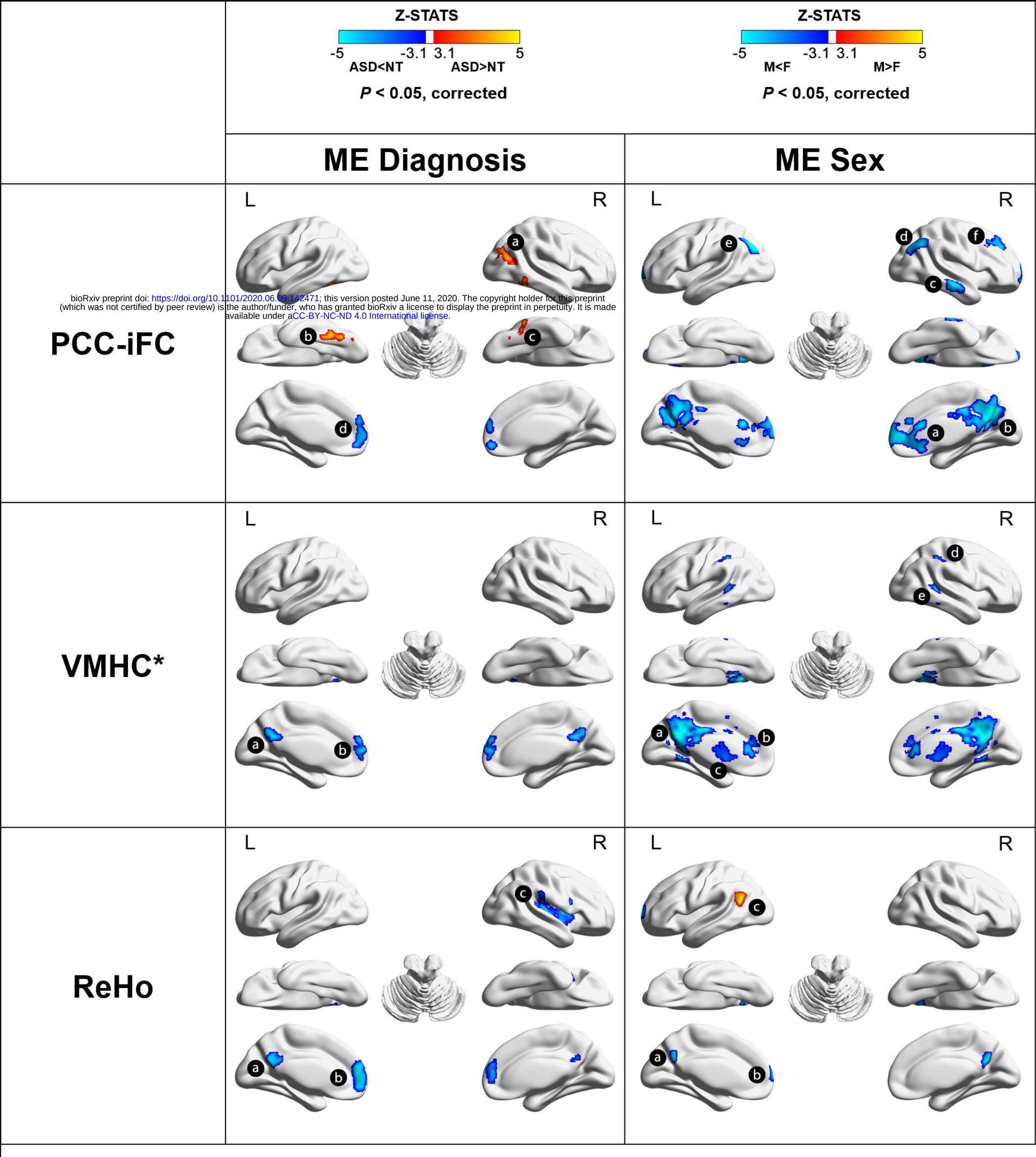
Abbreviations: ASD=autism spectrum disorder, NT=neurotypical, M=males, F=females, PCC-iFC=posterior cingulate cortex intrinsic functional connectivity (x=0, y=-53, z=26), VMHC=voxel-mirrored homotopic connectivity, ReHo=regional homogeneity, L=left, R=right, R+=replication based on same direction of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

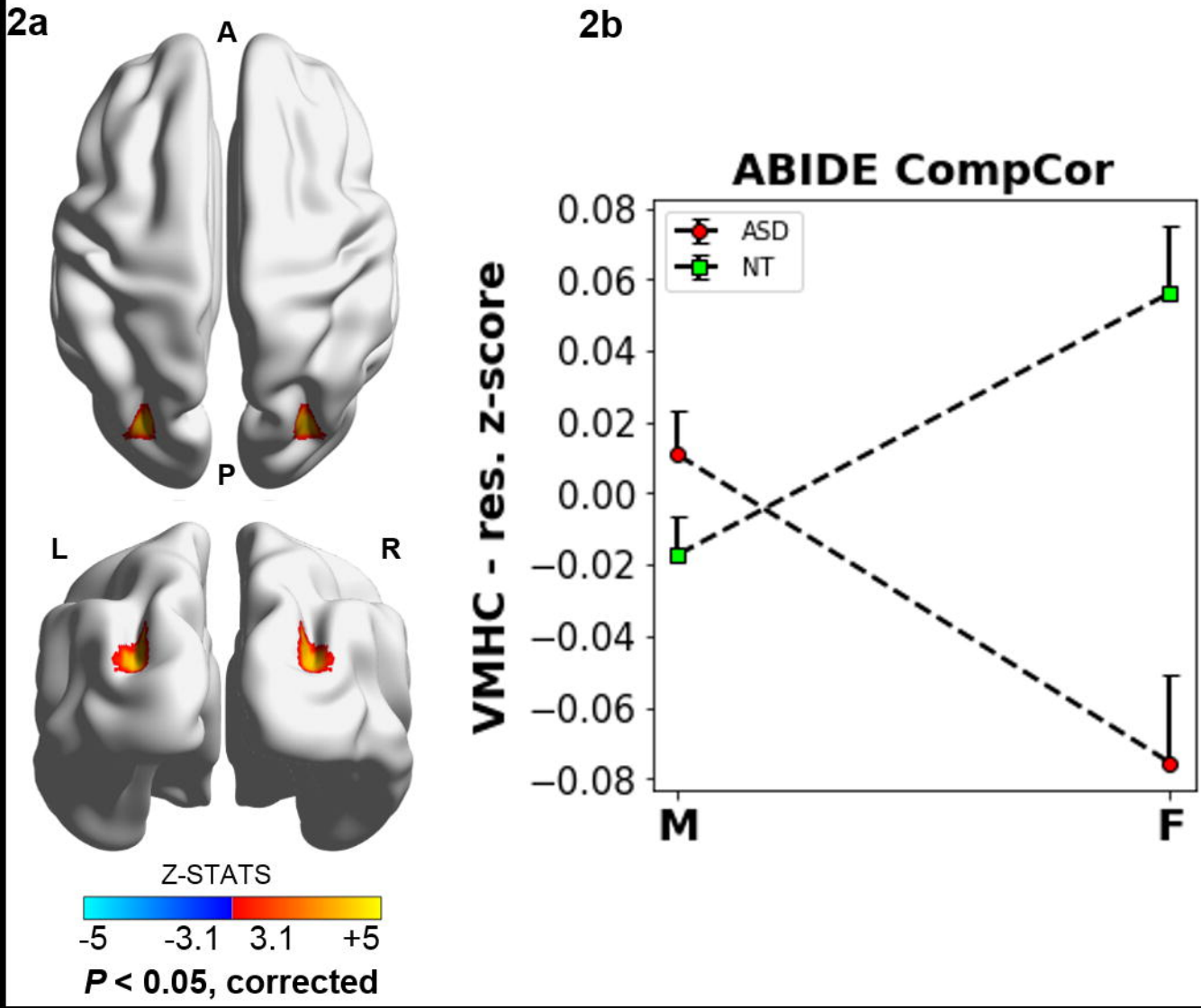
Table 1. Characterization of sample merged across ABIDE I and II

ABIDE I + II	Sites[a]	ASD M (N=362)	ASD F (N=82)	NT M (N=409)	NT F (N=166)	
	N	**Mean (SD) [Range]**	**Mean (SD) [Range]**	**Mean (SD) [Range]**	**Mean (SD) [Range]**	**Statistics**
Age	13	11.8 (2.6) [7-17.9]	11.7 (2.7) [7-18]	11.8 (2.6) [7.1-18.2]	11.4 (2.3) [7.8-17.4]	$F_{(3)}$=1.18, p=0.32
Full-Scale IQ[b]	13	106 (16.6) [72-148]	104 (16.3) [73-147]	112 (12.7) [73-148]	114 (12.7) [80-144]	$F_{(3)}$=19.24, p<0.001
Verbal IQ[c]	12	107 (17.9) [57-180]	105 (17.3) [62-145]	114 (13.5) [73-147]	114 (14.4) [83-146]	$F_{(3)}$=16.78, p<0.001
Performance IQ[d]	12	106 (17.0) [59-157]	104 (17.1) [67-148]	109 (14.2) [62-147]	109 (13.2) [79-145]	$F_{(3)}$=3.1, p=0.03
Mean FD	13	0.11 (0.07) [0.02-0.39]	0.13 (0.09) [0.02-0.39]	0.09 (0.06) [0.02-0.39]	0.09 (0.06) [0.02-0.38]	$H_{(3)}$=29.6, p<0.001
ADI-R						
Social[e]	11	19.7 (5.2) [4-30]	19.6 (5.5) [7-30]	-	-	$t_{(93)}$=0.14, p=0.89
Communication[f]	11	15.6 (4.5) [2-25]	15.2 (5.0) [4-24]	-	-	$t_{(92)}$=0.61, p=0.54
RRB[f]	11	6.0 (2.4) [0-13]	5.8 (2.5) [0-12]	-	-	$t_{(96)}$=0.51, p=0.61
ADOS-2						
Social-Affect[g]	11	9.1 (3.7) [1-20]	8.7 (3.2) [4-18]	-	-	$t_{(87)}$=0.97, p=0.33
RRB[h]	11	3.2 (1.8) [0-8]	2.8 (1.5) [0-5]	-	-	$t_{(93)}$=1.79, p=0.08
CSS total[i]	11	6.9 (2.1) [1-10]	6.8 (1.8) [2-10]	-	-	$t_{(93)}$=0.11, p=0.32
		N	**N**			**Statistics**
Comorbidity	5	99[j]	25[h]	-	-	$\chi^2_{(1)}$=0.2, p=0.66
Psychoactive Meds	10	112	26	-	-	$\chi^2_{(1)}$<0.01, p=0.99

Abbreviations: *ABIDE*: Autism Brain Imaging data exchange; *ADI-R* = Autism Diagnostic Interview-Revised; *ADOS-2* = Autism Diagnostic Observation Schedule-2; *ASD* = Autism Spectrum Disorder; *CSS* = Calibrated Severity Score*; F* = females; *IQ* = intellectual quotient; *M* = males; *Mean FD* = mean framewise displacement (Jenkinson *et al*., 2002); *NT* = neurotypical; RRB= restricted repetitive behaviors. **Notes**: [a]ABIDE I data collections: KKI, Leuven_2, NYU, OHSU, Pitt, SDSU, Stanford, UCLA_1, UM_1, and Yale. ABIDE II data collections: ABIDEII-GU_1, ABIDEII-KKI_1, ABIDEII-KKI_2, ABIDEII-NYU_1, ABIDEII-OHSU_1, ABIDEII-SDSU_1, ABIDEII-UCD_1 and ABIDEII-UCLA_1. KKI and ABIDEII-KKI_1, NYU and ABIDEII-NYU_1, SDSU and ABIDEII-SDSU_1, OHSU and ABIDEII-OHSU_1 and UCLA_1 and ABIDEII-UCLA_1 were merged into one site across ABIDE I and ABIDE II collections. [b]FIQ was available for 362 males with ASD (2 missing from UM_1, ABIDEII-SDSU_1), 81 females with ASD (1 missing from ABIDEII-GU_1), 407 neurotypical males (NT M) (3 missing from ABIDEII-GU_1 (N=1), UM_1 (N=2)) and all 166 NT females (NT F). [c]VIQ was available for 315 males with ASD (47 missing; KKI (N=14), ABIDEII-OHSU_1 (N=22), OHSU (N=9), ABIDEII-SDSU_1 (N=1); ABIDEII-UCLA_1 (N=1), 70 females with ASD (12 missing, ABIDEII-GU_1 (N=1), KKI (N=4), ABIDEII-OHSU_1 (N=7)), 351 NT M (59 missing; ABIDEII-GU_1 (N=1), KKI (N=23), OHSU (N=15), ABIDEII-OHSU_1 (N=20)), and 139 NT F (27 missing; KKI (N=8), ABIDEII-OHSU_1 (N=19)). [d]PIQ was available for 306 males with ASD (56 missing; ABIDEII-GU_1 (N=9), KKI (N=14), OHSU (N=9), ABIDEII-OHSU_1 (N=22), ABIDEII-UCLA_1 (N=1), UM_1, (N=1)), 67 females with ASD (15 missing; ABIDEII-GU_1 (N=4), KKI (N=4), ABIDEII-OHSU_1 (N=7)), 349 NT M (61 missing; ABIDEII-GU_1 (N=1), KKI (N=23), OHSU (N=15), ABIDEII-OHSU_1 (N=20), UM_1, (N=2)), 139 NT F (27 missing; KKI (N=8), ABIDEII-OHSU_1 (N=19)). [e]ADI-R Social scores were available for 317 males with ASD (45 missing; ABIDEII-GU_1 (N=1), Leuven_2 (N=10), NYU (N=3), ABIDEII-NYU_1 (N=1), SDSU (N=2), ABIDEII-UCD_1 (N=11), ABIDEII-UCLA_1 (N=14), UM_1, (N=2), Yale (N=3)) and 68 females with ASD (14 missing; ABIDEII-GU_1 (N=1), ABIDEII-KKI_1 (N=2), Leuven_2 (N=3), NYU (N=1), Pitt (N=1), ABIDEII-UCD_1 (N=3), ABIDEII-UCLA_1 (N=1), Yale (N=2)). [f]ADI-R Communication and RRB scores were available for 318 males with ASD (45 missing; ABIDEII-GU_1 (N=1), Leuven_2 (N=10), NYU (N=2), ABIDEII-NYU_1 (N=1), SDSU (N=2), ABIDEII-UCD_1 (N=11), ABIDEII-UCLA_1 (N=14), UM_1, (N=2), Yale (N=3)) and 68 females with ASD (14 missing; ABIDEII-GU_1 (N=1), ABIDEII-KKI_1 (N=2), Leuven_2 (N=3), NYU (N=1), Pitt (N=1), ABIDEII-UCD_1 (N=3), ABIDEII-UCLA_1 (N=1), Yale (N=2)). [g]ADOS-Gotham Social-Affect was available for 261 males with ASD (101 missing; ABIDEII-GU_1 (N=27), ABIDEII-KKI_1 (N=13), Leuven_2 (N=10), NYU (N=7), OHSU (N=11), Pitt (N=8), SDSU (N=8), Stanford (N=6), ABIDEII-UCLA_1 (N=5), UM_1 (N=6), Yale (N=1)) and 55 females with ASD (27 missing; ABIDEII-GU_1 (N=6), ABIDEII-KKI_1 (N=7), Leuven_2 (N=3), ABIDEII-OHSU_1 (N=1), Pitt (N=4),

Stanford (N=1), ABIDEII-UCD_1 (N=1), UCLA_1 (N=1), UM_1 (N=3)). [h]ADOS-Gotham RRB was available for 264 males with ASD (98 missing; ABIDEII-GU_1 (N=27), ABIDEII-KKI_1 (N=13), Leuven_2 (N=10), NYU (N=7), OHSU (N=11), Pitt (N=8), SDSU (N=8), Stanford (N=3), ABIDEII-UCLA_1 (N=5), UM_1 (N=6), Yale (N=1)) and 56 females with ASD (26 missing; ABIDEII-GU_1 (N=6), ABIDEII-KKI_1 (N=7), Leuven_2 (N=3), ABIDEII-OHSU_1 (N=1), Pitt (N=4), ABIDEII-UCD_1 (N=1), UCLA_1 (N=1), UM_1 (N=3)). [i]ADOS-Gotham calibrated severity scores (Gotham *et al.*, 2009) were available for 347 males with ASD (15 missing) and 77 females with ASD (5 missing). [j]Attention Deficit Hyperactivity Disorder (ADHD; N=63); anxiety disorder (N=22); Oppositional Defiant Disorder (ODD; N=17); mood disorder (N=11); Tourettes/Tics (N=6); Obsessive-Compulsive Disorder (OCD; N=6); enuresis (N=8); encopresis (N=4); developmental articulation disorder (N=1); developmental dyslexia (N=1); sensory integration disorder (N=1). [h]ADHD (N=17); anxiety disorder (N=7); ODD (N=10); mood disorder (N=2); OCD (N=2); enuresis (N=2); encopresis (N=1). The three group means were compared with ANOVA tests (or Kruskal-Wallis test in the case of non-parametric mean FD) followed by post-hoc pairwise t-test comparisons (or Mann-Whitney U-tests in the case of non-parametric mean FD) when statistically significant (significance cut-off set at $p<0.05$).

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M
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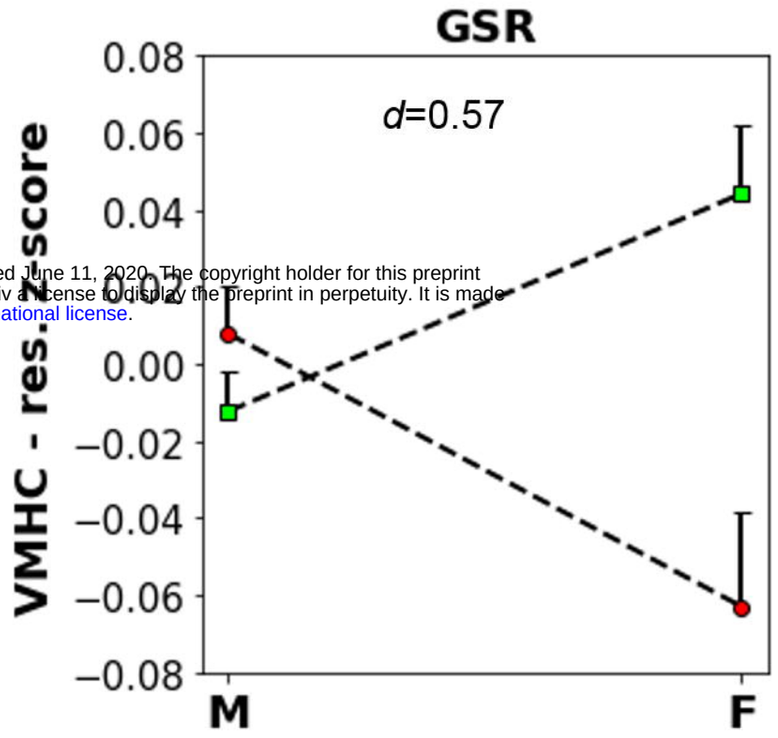
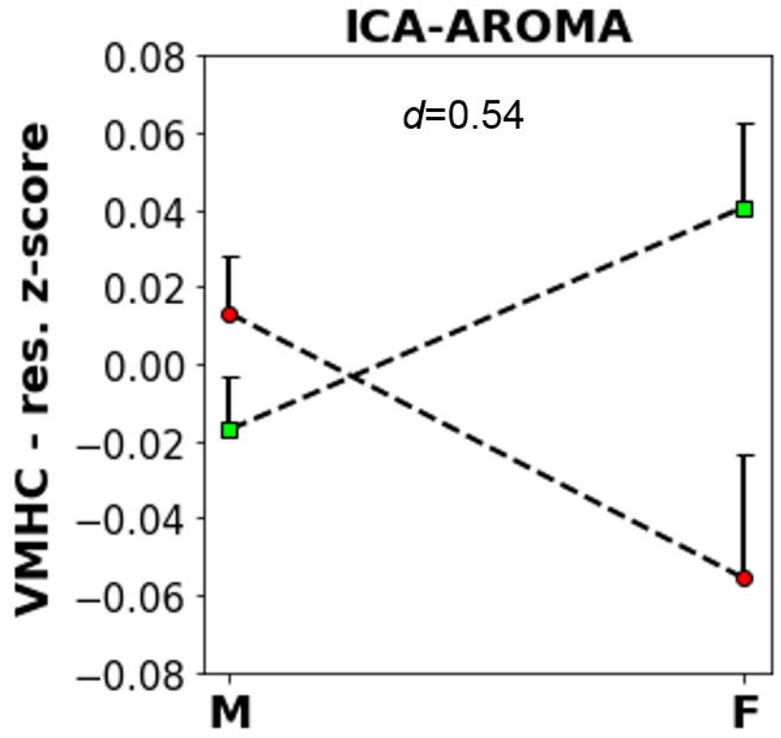
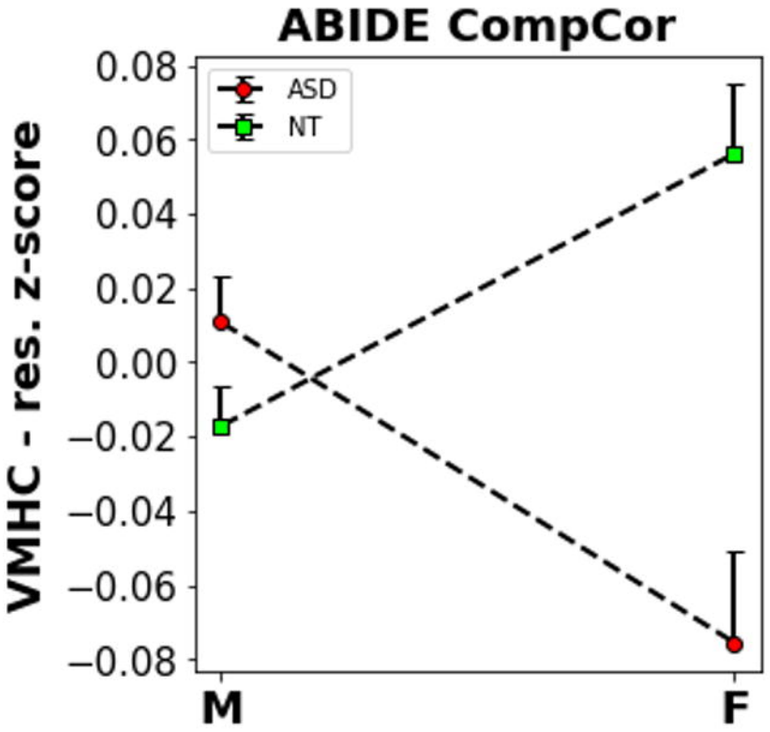
Processing Pipeline

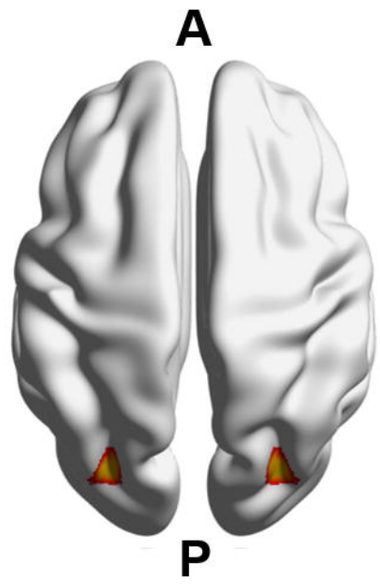
3a

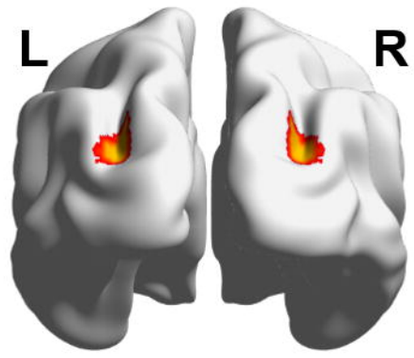
Robustness

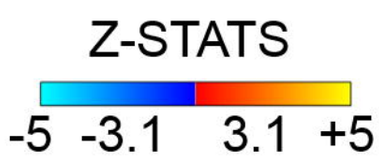










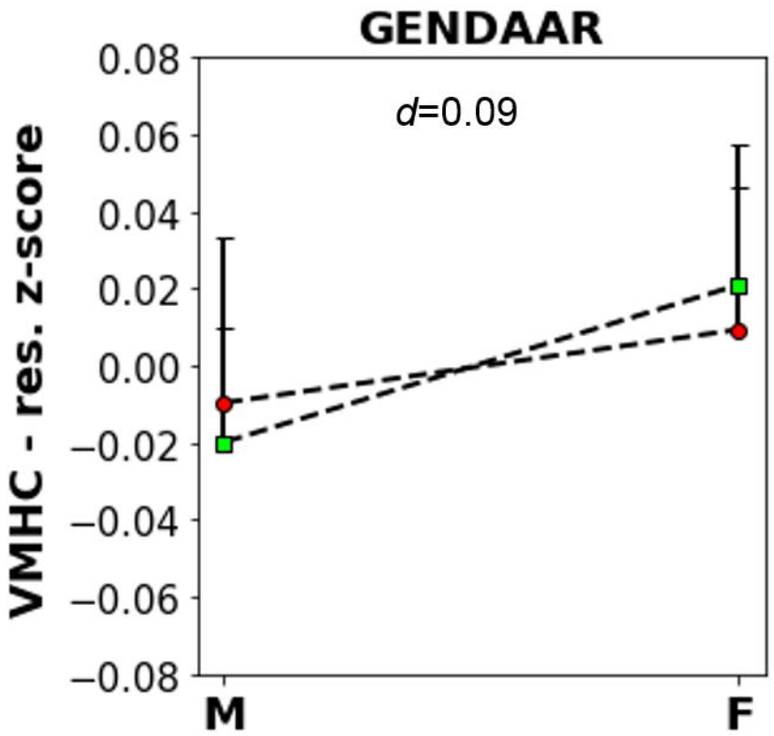


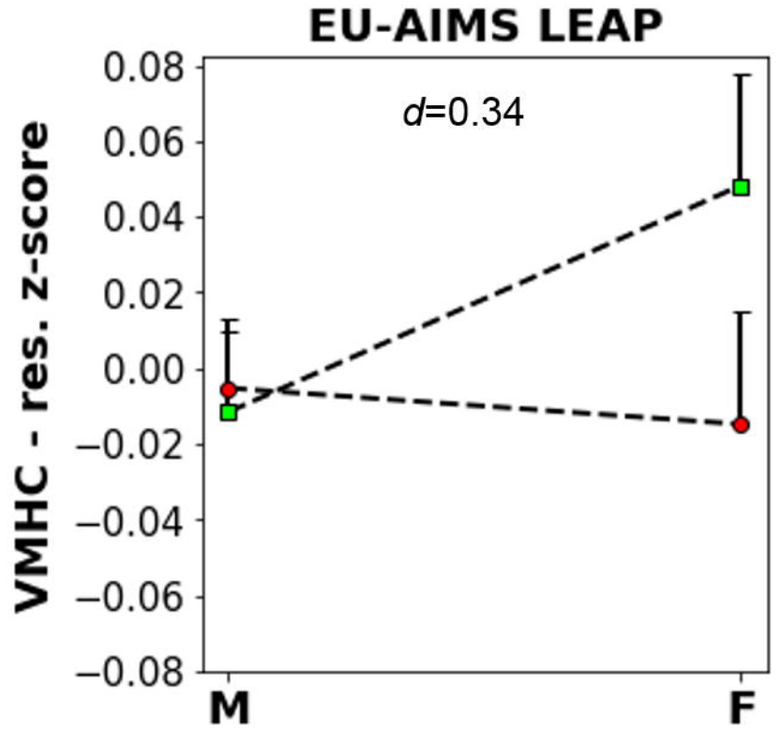
***P* < 0.05, corrected**

Sample

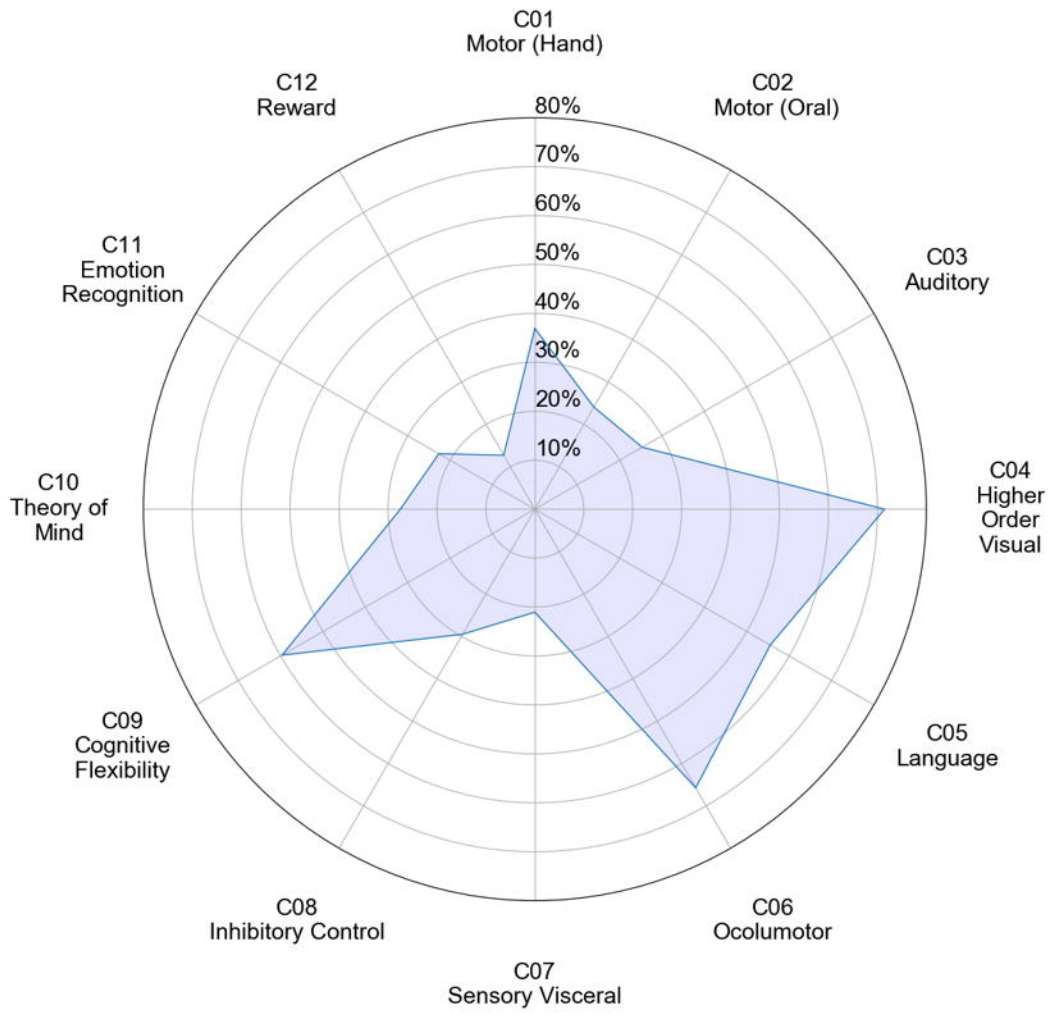
3b

Replicability

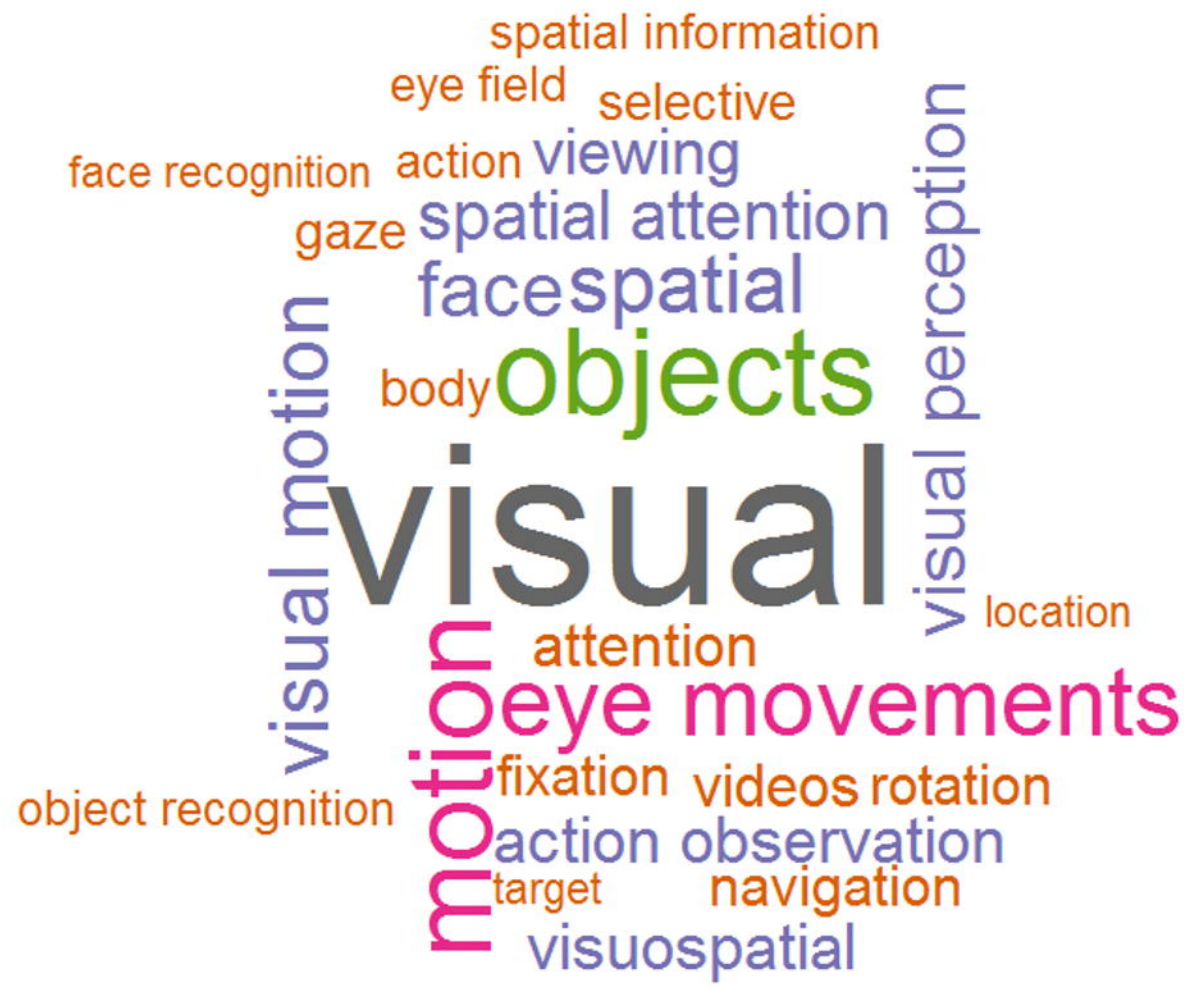




a) Overlap of VMHC cluster with cognitive ontology maps



b) Neurosynth terms correlation with VMHC cluster



c) Correlation between social-affect ADOS scores and VMHC cluster