Title

Sex Differences in the Amygdala Resting-State Connectome of Children With Autism Spectrum Disorder

Permalink

https://escholarship.org/uc/item/0rs8k2jd

Journal

Biological Psychiatry Cognitive Neuroscience and Neuroimaging, 5(3)

ISSN

2451-9022

Authors

Lee, Joshua K
Amaral, David G
Solomon, Marjorie
et al.

Publication Date

2020-03-01

DOI

10.1016/j.bpsc.2019.08.004

Peer reviewed

Sex Differences in the Amygdala Resting State Connectome of Children with Autism Spectrum Disorder

Joshua K. Lee[1,2], **David G. Amaral**[1,2], **Marjorie Solomon**[1,2], **Sally J. Rogers**[1,2], **Sally Ozonoff**[1,2], **Christine Wu Nordahl**[1,2]

[1]MIND Institute, University of California Davis School of Medicine, Sacramento, CA, USA;

[2]Department of Psychiatry and Behavioral Sciences, University of California Davis School of Medicine, Sacramento, CA, USA

Abstract

Background—Multifactorial liability models predict greater dissimilarity in the neural phenotype of autism spectrum disorder (ASD) in females than in males, while Gender Incoherence and Extreme Male Brain models predict attenuated sex differences in ASD. The amygdala is an informative target to explore these models because it is implicated in both the neurobiology of ASD and sex differences in typical development (TD).

Methods—We investigated amygdala resting-state functional connectivity in a cohort of 116 ASD (36 female) and 58 TD children (27 female) aged 2 to 7 years during natural sleep. First, multivariate distance matrix regression assessed global sex and diagnostic differences across the amygdala connectome. Second, univariate general linear models identified regions with mean connectivity differences.

Results—Multivariate distance matrix regression revealed greater differences between TD and ASD children in females than in males, consistent with multifactorial liability models, and attenuated sex differences in the left amygdala connectome of children with ASD in a pattern consistent with the Gender Incoherence model. Univariate analysis identified similar sex differences in dorsomedial and ventral prefrontal cortices, lingual gyrus, and the posterior cingulate cortex, but also that lower amygdala connectivity with superior temporal sulcus is observed across sexes.

Conclusions—We provide evidence that ASD in males and females manifests differently in the brain compared to sex-matched controls at the time of diagnosis and prior to the influence of compensatory mechanisms, consistent with multifactorial liability models, and that ASD is associated with reduced sex differences in a pattern consistent with gender incoherence models.

Corresponding Authors: Joshua K Lee, (916) 703-0360, jkilee@ucdavis.edu, Christine Wu Nordahl, (916) 703-0373, cnordahl@ucdavis.edu.

Keywords

amygdala; autism; imaging; connectome; sex; gender

Introduction

Autism Spectrum Disorder (ASD) is disproportionately diagnosed in males at a ratio of about 3–4 males for every female (1). The extant literature largely reflects this bias, and consequently, the neurobiology of ASD in females is not well characterized. Here we explored sex differences in the neurobiology of ASD during early childhood by investigating amygdala resting-state functional MRI (rsfMRI) connectivity. The amygdala is an interesting target for investigation because it exhibits both altered structure and function in ASD (2–5) and sex differences in typical development (6, 7).

The amygdala is widely implicated in the neurobiology of ASD. Postmortem research has reported differences in the number of amygdala neurons in ASD (2, 4), volumetric MRI research has reported amygdala enlargement in ASD (2, 8–11), and functional neuroimaging research has reported altered amygdala response and habituation to socio-emotional stimuli in ASD (12, 13). Altered amygdala rsfMRI connectivity has also been reported in individuals with ASD (3, 14–16), but samples have been predominantly male. To date, no analysis of sex differences in amygdala connectivity in ASD has been undertaken, which could be interesting given the robust sex differences in amygdala structure and function observed in typical development. The amygdala expresses particularly high concentrations of estrogen and androgen receptors (6, 17), exerting considerable influence over neurodevelopment beginning prenatally (18). Consistent with this, amygdala rsfMRI connectivity may follow different age-related trajectories in males and females in typical development (19) and divergent functional and neuronal responses to emotional face stimuli (20, 21). Structural MRI studies find increased volume in males across early childhood and adulthood, but females reach peak amygdala volume about 1.5 years sooner than males (7, 8, 22–24). Thus, the amygdala may be an especially informative brain region for exploring sex differences in ASD.

Resting-state fMRI is a useful tool to examine brain networks. In clinical populations, rsfMRI offers the advantage that it does not require performance of a task which may pose challenges that would preclude examination of many clinical populations. We examined sex differences in amygdala rsfMRI connectivity in 2 to 7-year-olds with ASD and typical development. We used two complementary analytical techniques: multivariate distance matrix regression (MDMR) assessed global sex and diagnostic differences in whole-brain amygdala rsfMRI connectivity maps (25–28) and univariate general linear models identified specific brain regions exhibiting mean differences in amygdala connectivity. Thus, MDMR can provide an overview of what is characteristic of the connectome while univariate analysis identifies the brain regions within that connectome exhibiting the most extreme differences.

To our knowledge, this is the first study to examine sex differences in rsfMRI connectivity in young children with ASD during a period of rapid brain growth and near the age of initial

diagnosis. We made several predictions based on existing literature and current theories regarding sex differences in the neurobiology of ASD. Our first set of predictions was based on the Extreme Male brain (EM) and Gender Incoherence (GI) theories (29–31). Both posit that atypical processes of masculinization and/or feminization affect the behavioral, physiological, and neurobiological features of ASD, resulting in attenuation of sex differences observed in typical development. However, EM and GI theories make different predictions in terms of the direction of differences from typically developing counterparts. While the EM hypothesis posits that both males and females with ASD are hyper-masculinized (29), the GI hypothesis posits that ASD in males is associated with *feminization* of features, and ASD in females is associated with *masculinization* of features (30, 32). Thus, shifts in amygdala connectivity in males and females with ASD towards TD males would be consistent with the EM hypothesis. On the other hand, shifts in amygdala connectivity toward the opposite TD sex would be consistent with the GI hypothesis (Figure 1A).

Our second set of predictions was based on the differential liability model (33), which posits that multiple genetic, biological, and environmental factors underlie total liability for an ASD diagnosis (i.e. etiologic load), and that females with ASD carry greater etiologic load than ASD males (33) (Figure 1B). Evidence for differential liability comes from genetic studies, and given that many affected genes are critical to neuronal function (34, 35), an extension of this model predicts that females with ASD will exhibit a higher liability of neural alterations as well. Thus, we predicted that differences in amygdala rsfMRI connectivity between ASD and TD females will be larger than between ASD and TD males.

Methods and Materials

Participants

One-hundred and sixteen children with ASD (36 female/80 male) and 58 TD children (27 female/31 male) aged 2 to 7 years were recruited as part of the UC Davis MIND Institute Girls with Autism Imaging of Neurodevelopment (GAIN) and the Autism Phenome Project (APP) longitudinal research programs. Children were enrolled at 2–3.5 years of age, and the design included three MRI annual time points separated with concurrent behavioral assessments at the first and third time points. The first time point with a quality rsfMRI was used (115 Time 1, 39 Time 2, 20 Time 3). A subset of male participants were included in a previous study (3). All children with ASD underwent diagnostic assessment at Time 1 and Time 3 by a licensed clinical psychologist specializing in ASD. Diagnostic assessments included the Autism Diagnostic Interview-Revised (ADI-R) (36) and the Autism Diagnostic Observation Schedule-2 (37). The ADOS calibrated severity score (CSS) was calculated to allow comparison different ADOS modules (38). Cognitive ability was assessed at Time 1 using the Mullen Scales of Early Learning (MSEL) (39) and the Differential Ability Scales-II (DAS-II) at Time 3 (40). No participant had known genetic (e.g. fragile X syndrome) or neurologic disorders, visual or hearing disability, or physical contraindication for MRI. TD controls had scores within 2 standard deviations of normative means on the MSEL and scores < 11) on the Social Communication Questionnaire (41). Participants taking any psychotropic medication were excluded from these analyses. Studies were approved by the

University of California, Davis Institutional Review Board. Parents or guardians provided informed consent.

Image Acquisition

T1-weighted magnetization-prepared rapid gradient echo images (MPRAGE; TR = 2170 ms; TE = 4.86 ms; slices: 192; matrix: 256 × 256, 1.0 mm isotropic voxels) and resting-state echo planar images (EPI; TR = 2000ms; TE = 27.0 ms; axial slices: 37; matrix: 64 × 64; 4.0 mm isotropic voxels) were acquired at the UC Davis Imaging Research Center in a 3T Siemens Tim Trio scanner with an 8-channel head coil. Image acquisition occurred during natural, nocturnal sleep (42). Sleep duration from sleep onset to the start of EPI acquisition was recorded (Table 1). The EPI acquisition occurred within the first 90 minutes of sleep, an epoch in which children are likely to be in non-rapid eye movement stage 3 sleep (43, 44).

Image Preprocessing

Resting-state images were preprocessed in the Configurable Pipeline for the Analysis of Connectomes (45; C-PAC v. 1.0.1; https://fcp-indi.github.io), which leverages tools from Analysis of Functional NeuroImages (AFNI; https://afni.nimh.nih.gov), FMRIB Software Library (FSL; https://fsl.fmrib.ox.ac.uk), and Advanced Normalization Tools (ANTs; http://stnava.github.io/ANTs). EPI images were time-shifted, and motion corrected. Volumes with frame-wise displacement greater than 0.25mm were scrubbed (46). Participants with fewer than 150 frames remaining after scrubbing were excluded. EPIs were co-registered to MPRAGE and normalized to MNI space. Mean white matter and cerebral spinal fluid, 24-degree Friston motion, and Global signal (GSR) (46) were entered as nuisance regressors. Supplemental analyses compared results using CompCor (47) in place of GSR. Data were then filtered (.008 < f < .08 Hz).

Analytic Approach

In all analyses, separate left and right amygdala connectivity maps (i.e. amygdala connectomes) were generated by extracting time series from amygdala seeds with all other voxels in the brain. Timeseries correlations were standardized using Fisher-Z transformations. Amygdala regions of interest were defined by a 1-mm erosion of the FSL Harvard-Oxford subcortical atlas binarized at the 50th percentile probability threshold.

Differences across the entire amygdala rsfMRI connectome were assessed using MDMR, while spatially localized differences were assessed with a univariate general linear model. Both MDMR and univariate analyses used the same factorial design: $Y \sim \beta_0 + \beta_1(\text{Sex}) + \beta_2(\text{Diagnosis}) + \beta_3(\text{Age}) + \beta_4(\text{Sex} \times \text{Diagnosis}) + \beta_5(\text{Sex} \times \text{Age}) + \beta_6(\text{Diagnosis} \times \text{Age}) + \beta_7(\text{Sex} \times \text{Diagnosis} \times \text{Age})$.

Multivariate Distance Matrix Regression—MDMR is a multivariate statistical method that allowed us to examine sex and diagnosis difference across whole-brain amygdala rsfMRI connectivity maps. MDMR is a robust multivariate technique appropriate to data in which responses (i.e. voxels) greatly outnumber participants (26), a scenario typical to neuroimaging data; consequently MDMR has found use in connectome-wide association

studies (27, 28). Here we used MDMR to test connectome-wide associations with *a priori* selected seed regions (left and right amygdala). No other seeds regions were examined.

To conduct the MDMR analysis, pair-wise distances between each participant's whole-brain amygdala connectivity map were computed using the Manhattan distance, a preferred distance in high-dimensional applications (48). The resulting distance matrix represented how dissimilar each individual's rsfMRI connectome was to every other individual's connectome. The relations between each explanatory variable and the distance matrix were estimated by apportioning the sums of squares of the Gower transformed distance matrix onto each variable and a residual term (26, 49). Test statistics were then permuted over 100,000 iterations. Interpretation of MDMR analysis was assisted by use of principal coordinate analysis (PCoA). Analogous to principal component analysis, PCoA attempts to capture most of the variation in a distance matrix using several dimensions, with the 1st dimension accounting for the most variation (49). The first several dimensions are typically interpreted via ordination plots and statistical analysis. The relative effect sizes of different brain regions contributing to the MDMR result were estimated (50) using 192 regions drawn from a brain parcellation (AICHA) (51). Analyses were conducted in R (v. 3.43) using the MDMR (v. 0.5.1) and Vegan (v 2.4–6) packages.

Univariate Analyses—General linear models were conducted on left and right amygdala resting-state statistical maps smoothed with a 6 mm Full-Width Half-Maximum Gaussian kernel. Correction for multiple comparisons employed Gaussian Random Field theory with a cluster forming threshold of $p < .001$ to control false positive rate (52, 53). Follow-up tests of marginal means were conducted using the R package lsmeans (v 2.26–3) with Tukey adjustment for multiple comparisons. In analyses identifying clusters exhibiting effects of ASD diagnosis common to both males and females, sex was coded as −1 and 1 for males and females respectively; this ensured that the intercept would be unbiased toward one sex over another, given the greater number of males with ASD in this sample.

Data Availability—Data from this study are available from the corresponding author and the senior authors upon reasonable request.

Results

Females and males with ASD were comparable across age, ADOS CSS, ADI-R Social Domain score, and DQ. Children with ASD had lower DQ than TD controls (Table 1). Motion was both minimal and not significantly different between groups. Sleep duration was similar between groups (Table 1).

Multivariate Distance Matrix Regression.

Left and right amygdala rsfMRI connectomes were investigated separately. For right amygdala, while the overall MDMR model was significant ($R^2 = .044$, $p = .045$), no individual effect parameters reached conventional significance (Table 2). For the left amygdala, the overall model was significant ($R^2 = .046$, $p = .011$), and a significant sex by diagnosis interaction was observed (Table 2). Pairwise comparison tests were conducted. Within diagnosis comparisons of sex (i.e. ASD females vs ASD males; TD females vs TD

males) revealed significant sex differences in the left amygdala connectome in TD (R^2 = .027, p = .005), but not in ASD (R^2 = .009, p = .47). Within sex comparisons of the effect of diagnosis (ASD females vs TD females; ASD males vs TD males) revealed significant diagnosis differences in females (R^2 = .022, p = .017), which explained nearly twice the variance in left amygdala connectivity than diagnosis difference in males (R^2 = .011, p = .052). Cross-sex, cross-diagnosis comparisons (ASD males vs TD females; ASD females vs TD males) revealed that left amygdala connectivity significantly differed between ASD males and TD females (R^2 = .014, p = .003), and marginally differed between ASD females and TD males (R^2 = .018, p = .081). Notably, both cross-comparisons explained a similar amount of variance, (see Figure S1 for plots of pairwise distances). We used PCoA to ascertain directionality of differences revealed in the sex by diagnosis interaction (Figure 2 A); the 1st PCoA axis exhibited a significant sex by diagnosis interaction (p =.01). Further information about the PCoA is provided in supplementary materials; see Figure S2A for a scree plot =eigenvalue decomposition and Figure S2B for group difference plots over the first four principal coordinates axes. Results were similar using data preprocessed with Compcor (47) in place of GSR (Figure S3A), suggesting that findings were not idiosyncratic to GSR. In addition to the sex by diagnosis interaction, the effect of diagnosis was moderated by age (Table 2), such that left amygdala connectivity was more dissimilar between ASD and TD groups in older children (R^2 = .017, p = .005) than in younger children (R^2 = .012, p = .52). Finally, relative effect sizes of each AICHA parcellation to left amygdala MDMR parameters are presented in Figure 2B. Visual inspection suggests that many brain regions contributed to the sex by diagnosis interaction, but particularly left amygdala–prefrontal connectivity.

Overall, analysis of the connectome revealed that sex differences in ASD were attenuated and shifted in the direction of the opposite TD sex, consistent with GI theory, and that ASD was associated with larger differences in females, consistent with the differential liability model.

Univariate General Linear Model Analyses

Multiple clusters revealed significant differences by sex, diagnosis, and age (Table 3). Four sex by diagnosis interaction clusters were identified (Table 3, Figure 3) between left amygdala and left dorsomedial prefrontal cortex (dmPFC), left ventral PFC, and left lingual gyrus, and between right amygdala and right posterior cingulate cortex. Post-hoc tests of differences in the marginal means evaluated at the mean age (]43.8 months) were conducted using the Tukey adjustment for multiple comparisons. Results of these tests are reported in Figure 3.

In general, two notable patterns were observed. First, in all four sex-by-diagnosis clusters, significant sex differences in TD controls were observed, but sex differences were attenuated in two of those clusters in ASD children. Second, compared to TD sex-matched counterpart, females with ASD exhibited larger and more significant differences than males with ASD (Figure 3). In all 4 clusters, significant or marginally significant diagnosis differences were observed in females, but significant diagnosis differences in males were observed only in left dmPFC and left lingual gyrus. The direction of differences in these two latter clusters

followed an opposite pattern in males and females (i.e. a crossed interaction pattern). For example, in the left dmPFC cluster, females with ASD exhibited higher connectivity than TD females, but this pattern was reversed in males, with ASD males exhibiting decreased connectivity relative to TD males. The pattern of results did not change when Compcor preprocessing was used instead of GSR (Figure S3B).

Thus far the reported univariate analyses emphasized the differences between males and females with ASD and their TD counterparts. However, ASD diagnosis was also associated with differences in amygdala rsfMRI connectivity across sex in several regions, including the superior temporal sulcus and supramarginal gyrus (Table 3). Reduced left and right amygdala connectivity in ASD was observed in three overlapping clusters along the entire length of the right superior temporal sulcus (Figure 4A). Reduced amygdala connectivity in ASD was also observed in right frontal operculum gyrus and left amygdala (Figure S4A). Increased amygdala connectivity in ASD was observed in three clusters (Table 3), including between left amygdala and left supramarginal gyrus (Figure 4B), left cerebral peduncle, and the anterior lobe of the cerebellum (Figure S4B). Alterations to amygdala connectivity in ASD exhibited age-related differences in multiple clusters, consistent with altered development that could result in increasing deviation from TD with age. Two clusters in right and two clusters in left superior temporal gyrus exhibited opposite age-related trajectories by diagnosis, such that age-related decreases in connectivity were observed in ASD (bs −.013, ts 2.11, ps .037), while age-related increases amygdala connectivity were observed in TD (bs .030, ts 3.22, ps .002) (Figure S5). These age-related differences suggest progressive hypoconnectivity in ASD during early childhood in the superior temporal gyrus, consistent with the hypo-connectivity observed in the superior temporal sulcus. Two clusters in left and right thalamus exhibited the opposite pattern of age-related trajectories in ASD and TD (Figure S6), such that age-related increases in right amygdala rsfMRI connectivity were observed in ASD (bs .027, ts 3.18, ps .002), but age-related decreases in TD (bs − .035, ts 2.72, ps .007).

Discussion

Within the emerging field investigating sex differences in ASD, there is interest in determining whether the etiology of ASD alters ongoing neurodevelopmental processes of sexual differentiation (29, 30, 54, 55) and the extent to which females and males with ASD differ from their sex-matched TD counterparts. Here we sought to characterize sex differences in amygdala rsfMRI connectivity in ASD—a brain region displaying both sex differences in typical development and alteration in ASD (3, 7). Multivariate and univariate analyses provided evidence that ASD is associated with attenuated sex differences in amygdala rsfMRI connectivity in a pattern that is consistent with the GI hypothesis (Figure 1A). We also found evidence that ASD is associated with greater differences in females than in males—consistent with multifactorial liability models hypothesizing female protective and/or male vulnerability factors which result in differential liability for ASD diagnosis (Figure 1B).

Multivariate analysis of sex differences in the amygdala rsfMRI connectome using MDMR revealed global sex differences in the left amygdala rsfMRI connectome of TD children but

these sex differences were attenuated in ASD. Many regions across the brain contributed in aggregate to this effect, including the frontal lobes, inferior and middle (but not superior) regions of the temporal lobe, the cuneus and lingual gyrus, and the putamen. On their own, differences in many of these regions would not be large enough to produce a significant finding, but by aggregating evidence across the connectome, MDMR allowed us to uncover the global trend. Univariate analysis complemented MDMR analysis by identifying clusters exhibiting the largest differences, revealing that left amygdala rsfMRI connectivity with the lingual gyrus and ventral PFC exhibited attenuated sex differences in ASD but robust sex differences in TD children. Consistent with our results, sex differences in lingual-amygdala connectivity have been reported in TD children (19) and hyperconnectivity has been reported in adolescents and adults with ASD (16). The lingual gyrus is involved in brain networks that process social stimuli, faces, and facial affect (20) and is frequently implicated in fMRI studies of ASD that use social stimuli (56). Ventral PFC is associated with social flexibility (57) and impaired cognitive control in ASD (58). Altogether, these data suggest atypical attenuation of sex differences in left amygdala rsfMRI connectivity, particularly in regions associated with regulating emotional social response. These findings also support the hypothesis that alteration in networks exhibiting sex differences in TD may be associated with at least some aspects of the ASD neuro-phenotype (59), consistent with the idea that sex differences reflect ongoing processes of neural development, which is regulated by many factors that are also associated with risk for ASD (18, 31, 34, 60, 61).

Our results suggest a global pattern of amygdala rsfMRI connectivity in males within ASD shifted away from TD males toward TD females, and connectivity in females shifted away from TD females towards TD males—a pattern which is most consistent with the Gender Incoherence model. Such shifts were most acutely observed in amygdala connectivity to left dmPFC and left lingual gyrus, in which females with ASD exhibited hyperconnectivity whereas males with ASD exhibited hypoconnectivity relative to their TD sex-matched counterparts. Opposite patterns of differences in males and females have been previously reported in univariate analysis (32, 59). However, as a practical matter, such mass univariate connectivity analyses tend to highlight the strongest forms of an interaction (i.e. fully crossed) because weaker forms fail to retain statistical significance after correction for multiple comparisons. The hazard is not subtle. The difference between EM masculinization (29) and GI models (30), for example, is the difference between a weaker and stronger form of a sex by diagnosis interaction. MDMR allowed us to assess the global pattern of sex differences and revealed a broader pattern of sex differences in the amygdala connectome consistent with gender incoherence in ASD.

We also found evidence supporting a multifactorial liability model of sex differences in ASD. Multifactorial liability models posit that multiple factors underlie total liability for an ASD diagnosis and that females with ASD carry greater etiologic load than ASD males (33–35), potentially via mechanisms of female protection or male vulnerability. Specifically, we predicted greater diagnosis differences in amygdala connectivity in females than in males. Consistent with prediction, we found larger within-sex differences in females than in males over the entire left amygdala rsfMRI connectome as well as in the specific clusters identified by univariate analysis, with significant differences between ASD and TD females in all four of sex by diagnosis interaction clusters, while significant differences between ASD and TD

males were observed in only two clusters. If the larger differences in amygdala connectomes between ASD and TD females are signatures reflecting female protective factors, the young age of our cohort increases the likelihood these protective factors reflect biological sex differences in neurodevelopment rather than gender-informed experiential factors (31).

Importantly, we also identified some similarities in the pattern of alterations across males and females. Both sexes exhibited reduced amygdala rsfMRI connectivity with the superior temporal sulcus (STS). Connectivity between amygdala and superior temporal sulcus has established anatomical connectivity (62–64), and plays a central role in social cognition (65), alterations of which are relevant to the core deficits of ASD (66, 67). Such sex-convergent alterations may be an especially important target for future research on the core etiologies of ASD.

The current research has several limitations. First, we did not investigate behavioral associations of amygdala rsfMRI connectivity differences as almost one quarter of participants did not have concurrent behavioral measures. Second, we did not directly measure sleep-stage in this study. Third, this study had a relatively small sample of females with ASD, underscoring the need for further research. Last, the current study used a cross-sectional design, and yet sex and gender may be intrinsic to how autism develops; longitudinal studies are needed to assess how sex and autism shape brain development within individuals.

In conclusion, this study provides evidence that ASD is associated with both sex-independent and sex-dependent alterations in amygdala functional connectivity observable near the age of first diagnosis. Sex-dependent alterations include both qualitative, i.e. attenuated sex differences in ASD consistent with the GI hypothesis, as well as quantitative differences in the magnitude of differences between males and females with ASD and their sex-specific TD. These findings suggest that brain regions and networks exhibiting sex differences in typical development (TD) underlie some aspects of the ASD neuro-phenotype (59), but that no single model can fully explain sex differences in ASD. Further investigation of sex-dependent differences in amygdala connectivity should be considered in relation to heterogeneity in behavioral symptoms and co-occurring conditions related to amygdala function, such as emotion regulation and anxiety.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

References

1. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. (2018): Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. MMWR Surveillance Summaries. 67: 1–23.

2. Schumann CM, Amaral DG (2006): Stereological Analysis of Amygdala Neuron Number in Autism. Journal of Neuroscience. 26: 7674–7679. [PubMed: 16855095]

3. Shen MD, Li DD, Keown CL, Lee A, Johnson RT, Angkustsiri K, et al. (2016): Functional Connectivity of the Amygdala Is Disrupted in Preschool-Aged Children With Autism Spectrum Disorder. Journal of the American Academy of Child & Adolescent Psychiatry. 55: 817–824. [PubMed: 27566123]

4. Avino TA, Barger N, Vargas MV, Carlson EL, Amaral DG, Bauman MD, Schumann CM (2018): Neuron numbers increase in the human amygdala from birth to adulthood, but not in autism. Proceedings of the National Academy of Sciences. 115: 3710–3715.

5. Hennessey T, Andari E, Rainnie DG (2018): RDoC-based categorization of amygdala functions and its implications in autism. Neuroscience & Biobehavioral Reviews. 90: 115–129. [PubMed: 29660417]

6. Österlund MK, Keller E, Hurd YL (2000): The Human Forebrain has Discrete Estrogen Receptor α Messenger Rna Expression: High Levels In The Amygdaloid Complex. Neuroscience. 95: 333–342. [PubMed: 10658612]

7. Ritchie SJ, Cox SR, Shen X, Lombardo MV, Reus LM, Alloza C, et al. (2018): Sex Differences in the Adult Human Brain: Evidence from 5216 UK Biobank Participants. Cerebral Cortex. 28: 2959–2975. [PubMed: 29771288]

8. Schumann CM (2004): The Amygdala Is Enlarged in Children But Not Adolescents with Autism; the Hippocampus Is Enlarged at All Ages. Journal of Neuroscience. 24: 6392–6401. [PubMed: 15254095]

9. Mosconi MW, Cody-Hazlett H, Poe MD, Gerig G, Gimpel-Smith R, Piven J (2009): Longitudinal Study of Amygdala Volume and Joint Attention in 2- to 4-Year-Old Children With Autism. Archives of General Psychiatry. 66: 509. [PubMed: 19414710]

10. Schumann CM, Barnes CC, Lord C, Courchesne E (2009): Amygdala Enlargement in Toddlers with Autism Related to Severity of Social and Communication Impairments. Biological Psychiatry. 66: 942–949. [PubMed: 19726029]

11. Nordahl CW (2012): Increased Rate of Amygdala Growth in Children Aged 2 to 4 Years With Autism Spectrum Disorders: A Longitudinal Study. Archives of General Psychiatry. 69: 53. [PubMed: 22213789]

12. Kleinhans NM, Johnson LC, Richards T, Mahurin R, Greenson J, Dawson G, Aylward E (2009): Reduced Neural Habituation in the Amygdala and Social Impairments in Autism Spectrum Disorders. American Journal of Psychiatry. 166: 467–475. [PubMed: 19223437]

13. Swartz JR, Wiggins JL, Carrasco M, Lord C, Monk CS (2013): Amygdala Habituation and Prefrontal Functional Connectivity in Youth With Autism Spectrum Disorders. Journal of the American Academy of Child & Adolescent Psychiatry. 52: 84–93. [PubMed: 23265636]

14. Ebisch SJH, Gallese V, Willems RM, Mantini D, Groen WB, Romani GL, et al. (2011): Altered intrinsic functional connectivity of anterior and posterior insula regions in high-functioning participants with autism spectrum disorder. Human Brain Mapping. 32: 1013–1028. [PubMed: 20645311]

15. von dem Hagen EAH, Stoyanova RS, Baron-Cohen S, Calder AJ (2013): Reduced functional connectivity within and between 'social' resting state networks in autism spectrum conditions. Social Cognitive and Affective Neuwscience. 8: 694–701.

16. Kleinhans NM, Reiter MA, Neuhaus E, Pauley G, Martin N, Dager S, Estes A (2016): Subregional differences in intrinsic amygdala hyperconnectivity and hypoconnectivity in autism spectrum disorder: Subregional amygdala connectivity in autism. Autism Research. 9: 760–772. [PubMed: 26666502]

17. Martini L, Milcangi RC (1991): Androgen Metabolism in the Brain. Journal of Seroid Biochemical Molecular Biology. 39: 819–828.

18. McCarthy MM (2016): Multifaceted origins of sex differences in the brain. Philosophical Transactions of the Royal Society B: Biological Sciences. 371: 20150106.

19. Alarcón G, Cservenka A, Rudolph MD, Fair DA, Nagel BJ (2015): Developmental sex differences in resting state functional connectivity of amygdala sub-regions. NeuroImage. 115:235–244.

20. Fusar-Poli P, Placentino A, Carletti F, Landi P, Allen P, Surguladze S, et al. (2009): Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. J Psychiatry Neurosci. 15.

21. Newhoff M, Treiman DM, Smith KA, Steinmetz PN (2015): Gender differences in human single neuron responses to male emotional faces. Frontiers in Human Neuwscience. 9. doi: 10.3389/fnhum.2015.00499.

22. Giedd JN, Vaituzis AC, Hamburger SD, Lange N, Rajapakse JC, Kaysen D, et al. (1996): Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: Ages 4–18 years. The Journal of Comparative Neurology. 366: 223–230. [PubMed: 8698883]

23. Uematsu A, Matsui M, Tanaka C, Takahashi T, Noguchi K, Suzuki M, Nishijo H (2012): Developmental Trajectories of Amygdala and Hippocampus from Infancy to Early Adulthood in Healthy Individuals. (Krueger F, editor) PLoS ONE. 7: e46970. [PubMed: 23056545]

24. Ruigrok ANV, Salimi-Khorshidi G, Lai M-C, Baron-Cohen S, Lombardo MV, Tait RJ, Suckling J (2014): A meta-analysis of sex differences in human brain structure. Neuroscience & Biobehavioral Reviews. 39: 34–50. [PubMed: 24374381]

25. Anderson MJ (2001): A new method for non-parametric multivariate analysis of variance. Austral Ecology. 26: 32–46.

26. Zapala MA, Schork NJ (2012): Statistical Properties of Multivariate Distance Matrix Regression for High-Dimensional Data Analysis. Frontiers in Genetics. 3. doi: 10.3389/fgene.2012.00190.

27. Shehzad Z, Kelly C, Reiss PT, Cameron Craddock R, Emerson JW, McMahon K, et al. (2014): A multivariate distance-based analytic framework for connectome-wide association studies. NeuroImage. 93: 74–94.

28. Satterthwaite TD, Vandekar SN, Wolf DH, Bassett DS, Ruparel K, Shehzad Z, et al. (2015): Connectome-wide network analysis of youth with Psychosis-Spectrum symptoms. Molecular Psychiatry. 20: 1508–1515. [PubMed: 26033240]

29. Baron-Cohen S (2002): The extreme male brain theory of autism. Trends in Cognitive Sciences. 6: 248–254. [PubMed: 12039606]

30. Bejerot S, Eriksson JM, Bonde S, Carlström K, Humble MB, Eriksson E (2012): The extreme male brain revisited: gender coherence in adults with autism spectrum disorder. British Journal of Psychiatry. 201: 116–123. [PubMed: 22500012]

31. Lai M-C, Lerch JP, Floris DL, Ruigrok ANV, Pohl A, Lombardo MV, Baron-Cohen S (2017): Imaging sex/gender and autism in the brain: Etiological implications: Imaging Sex/Gender and Autism in the Brain. Journal of Neuroscience Research. 95: 380–397. [PubMed: 27870420]

32. Alaerts K, Swinnen SP, Wenderoth N (2016): Sex differences in autism: a resting-state fMRI investigation of functional brain connectivity in males and females. Social Cognitive and Affective Neuroscience. 11: 1002–1016. [PubMed: 26989195]

33. Werling DM, Geschwind DH (2013): Sex differences in autism spectrum disorders: Current Opinion in Neurology. 26: 146–153. [PubMed: 23406909]

34. Gilman SR, Iossifov I, Levy D, Ronemus M, Wigler M, Vitkup D (2011): Rare de novo variants associated with autism implicate a large functional network of genes involved in formation and function of synapses. Neuron. 70: 898–907. [PubMed: 21658583]

35. Levy D, Ronemus M, Yamrom B, Lee Y, Leotta A, Kendall J, et al. (2011): Rare de novo and transmitted copy-number variation in autistic spectrum disorders. Neuron. 70: 886–897. [PubMed: 21658582]

36. Lord C, Rutter M, Le Couteur A (1994): Autism Diagnostic Interview-Revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. Journal of Autism and Developmental Disorders. 24: 659–685. [PubMed: 7814313]

37. Lord C, Risi S, Lambrecht L, Cook EH, Leventhal BL, DiLavore PC, et al. (2000): The Autism Diagnostic Observation Schedule–Generic: A Standard Measure of Social and Communication Deficits Associated with the Spectrum of Autism. 30: 205–223.

38. Gotham K, Pickles A, Lord C (2009): Standardizing ADOS Scores for a Measure of Severity in Autism Spectrum Disorders. Journal of Autism and Developmental Disorders. 39: 693–705. [PubMed: 19082876]

39. Mullen EM (1995): Mullen scales of early learning. AGS Circle Pines, MN.

40. Elliott CD (2007): Differential ability scales, 2nd ed. San Antonio, TX: Harcourt Assessment.

41. Rutter M, Bailey A, Lord C (2003): SCQ The Social Communication Questionnaire Torrance, CA: Western Psychological Services.

42. Nordahl CW, Simon TJ, Zierhut C, Solomon M, Rogers SJ, Amaral DG (2008): Brief Report: Methods for Acquiring Structural MRI Data in Very Young Children with Autism Without the Use of Sedation. Journal of Autism and Developmental Disorders. 38: 1581–1590. [PubMed: 18157624]

43. Bes F, Schulz H, Navelet Y, Salzarulo P (1991): The distribution of slow-wave sleep across the night: a comparison for infants, children, and adults. Sleep. 14: 5–12. [PubMed: 1811320]

44. Manning JH, Courchesne E, Fox PT (2013): Intrinsic connectivity network mapping in young children during natural sleep. NeuroImage. 83: 288–293.

45. Sikka S, Cheung B, Khanuja R, Ghosh S, Yan C, Li Q, et al. (2014): Towards automated analysis of connectomes: The configurable pipeline for the analysis of connectomes (c-pac)..

46. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE (2014): Methods to detect, characterize, and remove motion artifact in resting state fMRI. NeuroImage. 84: 320–341.

47. Behzadi Y, Restom K, Liau J, Liu TT (2007): A Component Based Noise Correction Method (CompCor) for BOLD and Perfusion Based fMRI. Neuroimage. 37: 90–101. [PubMed: 17560126]

48. Aggarwal CC, Hinneburg A, Keim DA (2001): On the Surprising Behavior of Distance Metrics in High Dimensional Space In: Van den Bussche J, Vianu V, editors. Database Theory — ICDT 2001. (Vol. 1973), Berlin, Heidelberg: Springer Berlin Heidelberg, pp 420–434.

49. Gower JC (1966): Some Distance Properties of Latent Root and Vector Methods Used in Multivariate Analysis. 53: 325–338.

50. McArtor DB, Lubke GH, Bergeman CS (2017): Extending multivariate distance matrix regression with an effect size measure and the asymptotic null distribution of the test statistic. Psychometrika. 82: 1052–1077. [PubMed: 27738957]

51. Joliot M, Jobard G, Naveau M, Delcroix N, Petit L, Zago L, et al. (2015): AICHA: An atlas of intrinsic connectivity of homotopic areas. Journal of Neuroscience Methods. 254: 46–59. [PubMed: 26213217]

52. Woo C-W, Krishnan A, Wager TD (2014): Cluster-extent based thresholding in fMRI analyses: Pitfalls and recommendations. NeuroImage. 91: 412–419. [PubMed: 24412399]

53. Roiser JP, Linden DE, Gorno-Tempini ML, Moran RJ, Dickerson BC, Grafton ST (2016): Minimum statistical standards for submissions to Neuroimage: Clinical. NeuroImage: Clinical. 12: 1045–1047. [PubMed: 27995071]

54. Carter C (2007): Sex differences in oxytocin and vasopressin: Implications for autism spectrum disorders? Behavioural Brain Research. 176: 170–186. [PubMed: 17000015]

55. Ferri SL, Abel T, Brodkin ES (2018): Sex Differences in Autism Spectrum Disorder: a Review. Current Psychiatry Reports. 20. doi: 10.1007/s11920-018-0874-2.

56. Di Martino A, Ross K, Uddin LQ, Sklar AB, Castellanos FX, Milham MP (2009): Functional Brain Correlates of Social and Non-Social Processes in Autism Spectrum Disorders: an ALE Meta-Analysis. Biol Psychiatry. 65: 63–74. [PubMed: 18996505]

57. Nelson EE, Guyer AE (2011): The Development of the Ventral Prefrontal Cortex and Social Flexibility. Dev Cogn Neurosci. 1: 233–245. [PubMed: 21804907]

58. Solomon M, Ozonoff SJ, Ursu S, Ravizza S, Cummings N, Ly S, Carter CS (2009): The neural substrates of cognitive control deficits in autism spectrum disorders. Neuropsychologia. 47: 2515–2526. [PubMed: 19410583]

59. Lai M-C, Lombardo MV, Suckling J, Ruigrok ANV, Chakrabarti B, Ecker C, et al. (2013): Biological sex affects the neurobiology of autism. Brain. 136: 2799–2815. [PubMed: 23935125]

60. Chen C, Van Horn JD, GENDAAR Research Consortium (2017): Developmental neurogenetics and multimodal neuroimaging of sex differences in autism. Brain Imaging and Behavior. 11: 38–61. [PubMed: 26781567]

61. Mitra I, Tsang K, Ladd-Acosta C, Croen LA, Aldinger KA, Hendren RL, et al. (2016): Pleiotropic Mechanisms Indicated for Sex Differences in Autism. (Flint J, editor) PLOS Genetics. 12: e1006425. [PubMed: 27846226]

62. Amaral DG, Price JL (1984): Amygdalo-cortical projections in the monkey (Macaca fascicularis). The Journal of Comparative Neurology. 230: 465–496. [PubMed: 6520247]

63. Pitcher D, Japee S, Rauth L, Ungerleider LG (2017): The Superior Temporal Sulcus Is Causally Connected to the Amygdala: A Combined TBS-fMRI Study. J Neurosci. 37: 1156–1161. [PubMed: 28011742]

64. Brothers L, Ring B, Kling A (1990): Response of neurons in the macaque amygdala to complex social stimuli. Behavioural Brain Research. 41: 199–213. [PubMed: 2288672]

65. Overwalle FV (2009): Social cognition and the brain: A meta-analysis. Human Brain Mapping. 30: 829–858. [PubMed: 18381770]

66. Redcay E (2008): The superior temporal sulcus performs a common function for social and speech perception: Implications for the emergence of autism. Neuroscience & Biobehavioral Reviews. 32: 123–142. [PubMed: 17706781]

67. Patriquin MA, DeRamus T, Libero LE, Laird A, Kana RK (2016): Neuroanatomical and neurofunctional markers of social cognition in autism spectrum disorder. Human Brain Mapping. 37: 3957–3978. [PubMed: 27329401]
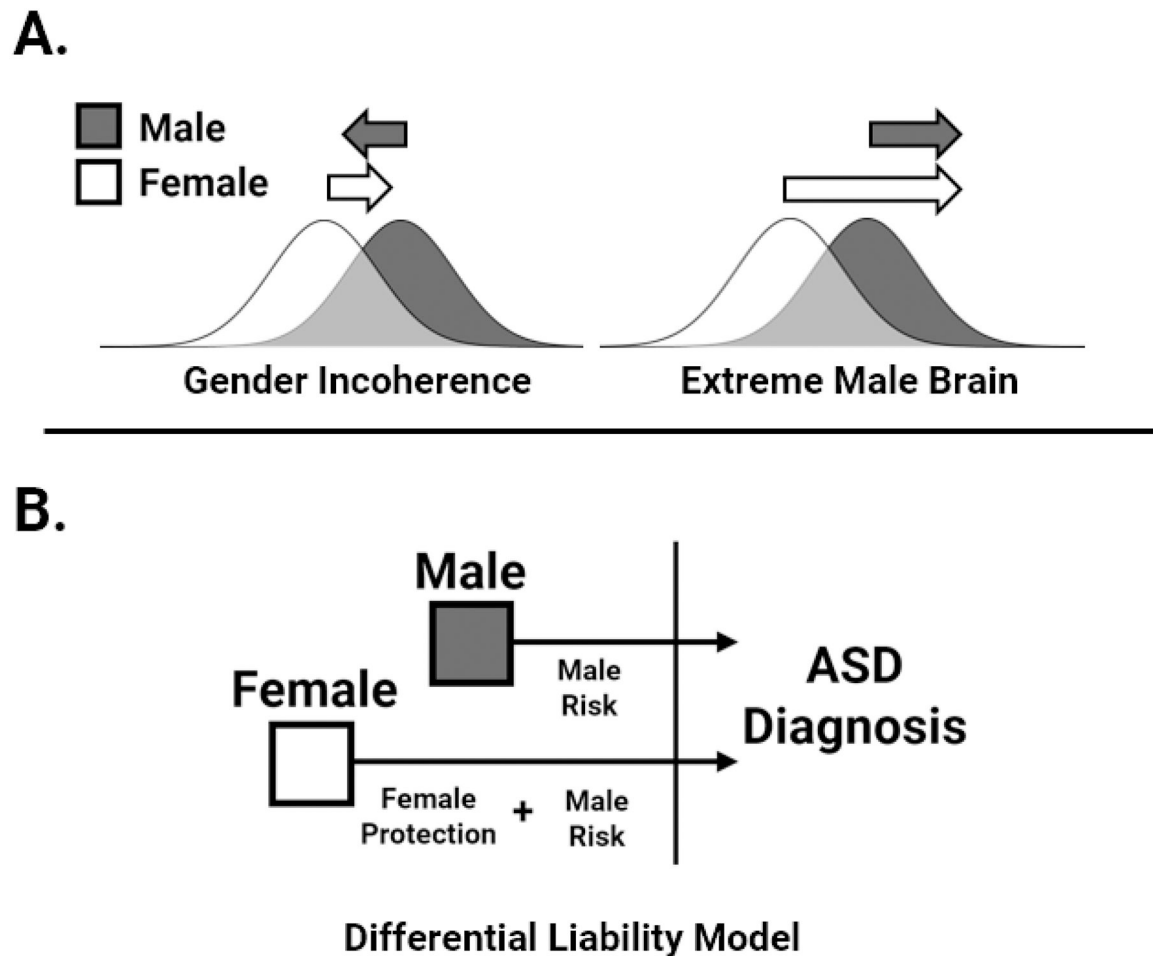
Figure 1.
A. The Gender Incoherence model of ASD, Bejerot et al., (2012) proposes that sex differences are altered in ASD, such that, on a normative male–female dimension, ASD is characterized by a shift in the distribution of some unknown subset of features towards the opposite gender/sex. The Extreme Male Brain also proposes that sex differences are altered in ASD, but characterized by a shift toward the extremes of TD males. Consequently, both Gender Incoherence and Extreme Male Brain predict that mean differences in the distributions of males and females with ASD is reduced and have greater overlap, albeit by differentially directed shifts relative to their sex-matched TD counterparts. **B.** The Multifactorial Differential Liability model for ASD (Werling and Geschwind, 2013) posits that multiple genetic, biological, and environmental factors underly total liability for ASD. Male-specific risk factors shift the male population towards, and female-specific protective factors shift the female population away from a single liability threshold. Consequently, females will, on average, be further away from the threshold than males, resulting in fewer ASD diagnoses for females. Given that many of the genetic and biological liability factors for ASD are associated with key aspects of brain development and function, an extension of the differential liability model is that differences in brain networks (e.g., the amygdala connectome) between TD and ASD females will be greater than between ASD and TD males.

Figure 2.
Sex and diagnostic differences in the left amygdala resting-state functional connectome. **A.** Left amygdala resting-state connectome significantly differed between typically developing (TD) males and females, but not significantly between males and females with an ASD diagnosis, as depicted in this ordination plot of the first two principal coordinate analytic (PCoA) decompositions of the left amygdala resting-state functional connectome's Manhattan distance matrix. The 1st PCoA axis exhibited a significant sex by diagnosis interaction ($p = .01$, see also Figure S3 for additional details). The reader should note that Multivariate Distance Matrix Regression (MDMR) does not utilize dimension reduction (i.e. PCoA) and thus this ordination may not fully capture all the multi-dimensional differences contributing to the MDMR result. Ellipses indicate 95% confidence intervals for centroid locations on the first two PCoA axes. **B.** Plot of the estimated relative effect sizes of brain regions contributing to MDMR model effects for the left amygdala resting-state functional connectome.
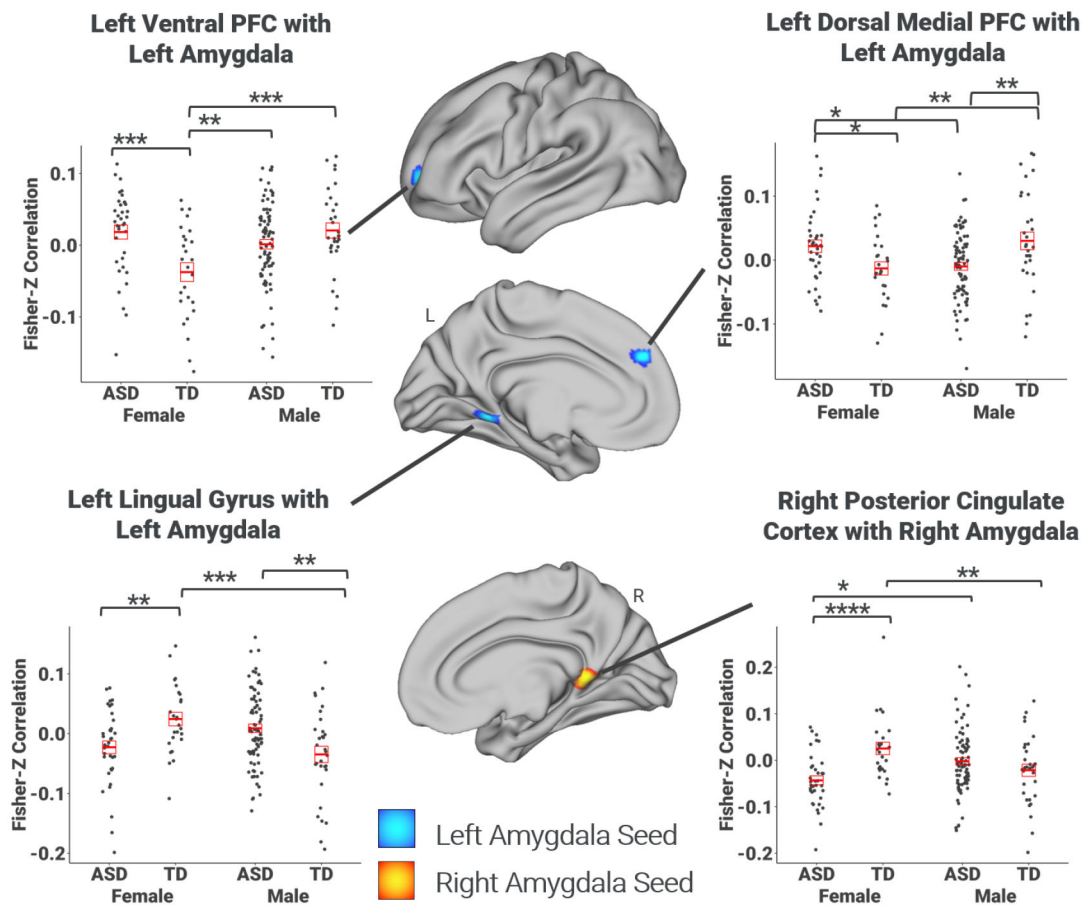
Figure 3.
Clusters exhibiting significant sex by diagnosis interaction F effects from univariate general linear models. Tests of predicted marginal means were conducted within each cluster using the Tukey adjustment for multiple comparisons. Error bars represent standard error. Significance codes: * <.05, ** <.01, <.001, ****<.0001.

Figure 4.
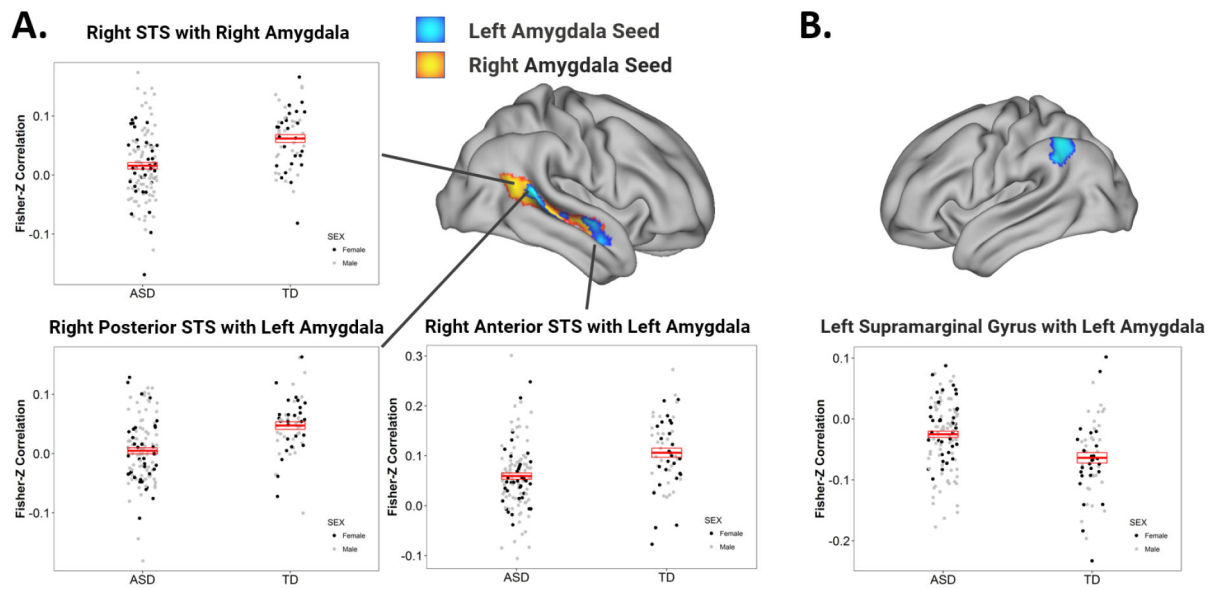Clusters exhibiting significant differences by diagnosis (in both males and females) in a univariate general linear model of amygdala resting-state functional connectivity. A. Lower left and right amygdala resting-state fMRI connectivity with right superior temporal sulcus (STS) in ASD. B. Greater left amygdala resting-state fMRI connectivity with left supramarginal gyrus in ASD. Error bars represent standard error.

Table 1.

Sample Behavioral and Scanning Characteristics

	Female		Male		
Measure	**ASD**	**TD**	**ASD**	**TD**	***p***
N	36	26	80	31	
Age (Months)	44.6 (11)	41.1 (7.7)	44.6 (12)	42.2 (12)	*p*s .28
ADOS-CSS	7.31 (1.9)	–	7.52 (1.7)	–	*p* = .53
ADI-R Social	18.3 (3.9)	–	16.9 (4.1)	–	*p* = .09
DQ	66.9 (24)	107 (11)	66.0 (23)	103 (12)	Sex: *p*s .55
					Dx: *p*<1e-12
Framewise Displacement (mm)	.126 (.05)	.120 (.05)	.114 (.04)	.120 (.04)	*p*s .19
Proportion Frames Scrubbed	.068 (.06)	.060 (.06)	.051 (.04)	.044 (.05)	*p*s .23
Sleep Duration (minutes)	51.5 (15)	46.5(12)	51.1(12)	48.4(12)	*p*s .13

Note: Means and Standard deviations = $M(SD)$; reported p-values are for F-tests of sex, or sex by diagnosis differences; ADOS-CSS= Autism Diagnostic Observation Schedule Calibrated Severity Score; ASD Autism Spectrum Disorder; ADI-R Autism Diagnostic Interview; TD = Typically Developing; Dx = Diagnosis. Means and Standard deviations = M(SD); ADOS and DQ characteristics report concurrent scores for Time 1 and Time 3 participants. Concurrent scores were unavailable for Time 2 participants (23 ASD, 16 TD), thus Time 1 were used. Mean framewise displacement computed using method presented in Power et al (2012); Sleep duration is time from beginning of sleep to start of resting-state scan.

Table 2.

Multivariate Distance Matrix Regression of Amygdala Connectomes

Effect	Right Amygdala				Left Amygdala			
	Statistic	R^2	p		Statistic	R^2	p	
(Omnibus)	.047	.044	.045	*	.048	.046	.011	*
Age	.008	.007	.057		.006	.006	.429	
Sex	.006	.006	.556		.008	.008	.011	*
Dx	.006	.006	.279		.008	.008	.026	*
Sex × Dx	.006	.005	.768		.007	.007	.049	*
Age × Sex	.006	.006	.346		.006	.005	.635	
Age × Dx	.007	.007	.114		.008	.008	.024	*
Age × Dx × Sex	.006	.006	.512		.005	.005	.981	

Notes:

*
p .05;

Statistics computed over 100,000 permutations; Dx=Diagnosis.

Table 3.

General Linear Model of Amygdala Resting-State Functional Connectivity

Effect	Amygdala	Region	MNI			Cluster Voxels	Cluster *p* Value	Relation
			X	Y	Z			
Sex × Dx	Right	Right Posterior Cingulate Cortex	10	−48	6	124	.000003	ASD: F<M
								TD: F>M
								Female: ASD<TD
								Male: ASD~TD
	Left	Left Lingual Gyrus	−10	−50	−10	84	.0008	ASD: F~M
								TD: F>M
								Female: ASD<TD
								Male: ASD>TD
		Left Ventral PFC	−20	56	−4	50	.019	ASD: F~M
								TD: F<M
								Female: ASD>TD
								Male: ASD~TD
		Left Dorsal Medial PFC	−6	44	28	60	.007	ASD: F>M
								TD: F<M
								Female: ASD>TD
								Male: ASD<TD
Dx × Age	Right	Right Thalamus	18	−28	0	159	4.9e-5	ASD: ↑ Age; TD: ↓ Age
		Left Thalamus	−10	−28	14	74	.010	ASD: ↑ Age; TD: ↓ Age
		Right Superior Temporal Gyrus	50	−8	−8	82	.0058	ASD: ↓ Age; TD: ↑ Age
		Left Superior Temporal Gyrus	−50	−12	2	251	4.2e-7	ASD: ↓ Age; TD: ↑ Age
	Left	Right Superior Temporal Pole	46	6	−12	236	1.2e-7	ASD: ↓ Age; TD: ↑ Age
		Left Superior Temporal Gyrus	−46	−16	−4	89	.0013	ASD: ↓ Age; TD: ↑ Age
Dx	Right	Right Superior Temporal Sulcus	52	−40	4	908	1.1e-22	TD > ASD
	Left	Right Superior Temporal Sulcus	58	−8	−12	56	.022	TD > ASD
		Right Superior Temporal Sulcus	46	−44	8	144	.00004	TD > ASD
		Right Frontal Operculum Gyrus	46	−2	6	135	.00006	TD > ASD
		Left Supramarginal Gyrus	−50	−54	42	268	2.9e-8	ASD > TD
		Left Cerebral Peduncle	−20	−14	−10	61	.015	ASD > TD
		Anterior Lobe of the Cerebellum	−2	−48	−20	76	.0043	ASD > TD

Notes: Correction for multiple comparisons of F-tests was performed using random field theory with a cluster forming threshold of *p* = .001; Parameter tests were performed on the mean correlation of a cluster in each individual using R. Dx=Diagnosis; M= Male; F= Female; ↑ = Significant Increase. ↓ = Significant Decrease; ~ = Not significant difference; w/ = with.