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Default mode hypoconnectivity underlies a sex-related autism spectrum

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Abstract

Background Females and males differ significantly in the prevalence and presentation of autism spectrum conditions. One theory of this effect postulates that autistic traits lie on a sex-related continuum in the general population, and autism represents the extreme male end of this spectrum. This theory predicts that any feature of autism in males should 1) be present in autistic females, 2) differentiate between the sexes in the typical population and 3) correlate with autistic traits. Here we tested these three predictions for default mode network (DMN) hypoconnectivity during resting state, one of the most robustly found neurobiological differences in autism.

Methods We analyzed a primary dataset of adolescents (n=121, 12-18 years) containing a relatively large number of females, and a replication multisite dataset including children, adolescents and adults (n=980, 6-58 years). We quantified the average connectivity between DMN regions, and tested for group differences and correlation with behavioral performance using robust regression.

Results We found significant differences in DMN intra-connectivity 1) between female controls and females with autism ($p = .001$, primary dataset; $p = .009$, replication dataset), and 2) between female controls and male controls ($p = .036$, primary dataset; $p = .002$, replication dataset). We additionally found 3) a significant correlation between DMN intra-connectivity and performance on a mentalizing task ($p = .001$) in the primary dataset.

Conclusions Collectively, these findings provide the first evidence for DMN hypoconnectivity as a behaviorally relevant, neuroimaging phenotype of the sex-related spectrum of autistic traits, of which autism represents the extreme case.

Text

Introduction

The strikingly high male to female prevalence ratio is one of the most obvious and robust characteristics of autism spectrum conditions (ASC) (1–3). While it is not known whether this bias reflects differential rates of diagnosis or genuine sex differences in prevalence (4; 5), the link between autism and the male sex is common in pervasive public stereotypes and originates with the first descriptions of these conditions. Asperger, having never encountered a female patient, informally described his eponymous syndrome as an "extreme variant of male intelligence" (6). A later formulation of this original observation noted population-level differences between the sexes in systemizing (a tendency to think mechanistically and logically, to perceive patterns and systems) and empathizing (the ability to identify and affectively share the emotional states of others), which are respectively stronger and weaker in ASC (7; 8). As males typically show an attenuated version of the same trend (greater systemizing and lower empathizing), these observations have led to the hypothesis that autistic traits exist on a continuum in the typical population (a prediction borne out by studies in genetics (9)) and that ASC represent an extreme form of the typical male brain (7; 8; 10; 11).

The neurobiological underpinnings of this framework have received little attention. Most neuroimaging studies have focused on identifying neurobiological features of autism, usually in predominantly male populations. The parsimonious prediction generated by the framework is that such neurobiological differences in ASC would further reflect the 'extreme' position of these individuals on the spectrum on which typical males and females differ. More specifically, a robust neurobiological feature of autism in males, would 1) be

similarly present in females with ASC, 2) show sex-specific differences in the typically developing population, and 3) correlate with autistic behavioral traits. However, these predictions have not been previously tested.

Rather than examining brain areas in isolation, much autism research has focused on functional connectivity patterns between brain regions (12). Functional connectivity is defined as the statistical association between pairs of brain regions, and may be inferred across a range of spatial and temporal scales, with a variety of measures. In the present article we focus on the most common operationalization of this concept in human neuroimaging: the computation of functional MRI connectivity using Pearson correlation (13). The default mode network (DMN) has been of particular interest in ASC due to its putative role in mentalizing and social cognition (14–18). This network, a group of brain regions which reduce their activity during cognitive processing, fail to deactivate in ASC (19–21). Altered functional connectivity between DMN regions at rest (22–29) and during tasks (30) is among the most commonly reported functional connectivity findings in ASC. Differences in functional connectivity within this network have been found across a range of methods including independent component analysis (22; 24), ROI or seed-based analyses (23; 26–29) and graph-theoretical analyses (25; 30). Furthermore, these differences correlate with core ASC symptoms (22; 23; 27; 28), and constitute an endophenotype (21; 30) – a genetically mediated biomarker (31) which distinguishes biological relatives of people with ASC from other members of the population. However the current literature on sex differences in DMN connectivity is sparse and inconsistent (32–34). Alteration of DMN connectivity thus represents a natural target for investigation of the hypothesized sex-related spectrum of autistic traits.

Here, we leveraged a primary dataset with a relatively large number of females with ASC and female siblings of individuals with ASC, and a replication multisite dataset, to

robustly test the predictions made by this framework. All participants were scanned during resting state, that is, a condition of lying still and quietly, unengaged in cognitive tasks. We specifically tested whether weaker functional connectivity between regions of the DMN is 1) a feature and endophenotype for autism in females, as has previously been shown in males with autism, 2) present in males relative to females in the typically developing population and 3) correlates with decreased mentalizing ability, typically affected in autism. We also tested the specificity of DMN hypoconnectivity by leveraging a positive control dataset of participants with a distinct psychiatric condition, major depressive disorder.

Materials and methods

Primary dataset: the Cambridge Family Study of Autism (CFSA)

The Cambridge Family Study of Autism (CFSA) comprises resting state and task scans from control females (n=20), control males (n=20), females with ASC (n=16), males with ASC (n=35) and non-affected female (n=30) and male (n=13) siblings of subjects with ASC (21; 30; 35–39). We used only the resting state scans from this sample for analysis. All participants were aged 12-18 and had no history of psychotropic drug usage and no other documented psychiatric conditions. Diagnostic status of the ASC group was confirmed with the ADOS-G and ADI-R, gold-standard tools in autism diagnosis (40; 41). See Supplemental Information (SI) 1 for full demographic details of all participants.

Replication dataset: Autism Brain Imaging Data Exchange (ABIDE)

To test the reproducibility of findings from the CFSA data, we analyzed resting state scans from 408 males with ASC, 428 control males, 55 females with ASC and 89 control females, obtained from the Autism Brain Imaging Data Exchange (ABIDE:

http://fcon_1000.projects.nitrc.org/indi/abide/) (42). These data were collected from 15 imaging sites, and participants spanned a wide age range between 6-58 years old. However, 456 (47%) participants were in the same age range (12-18 years old) as the CFSA participants. This replication dataset offers a considerable increase in statistical power, at the expense of a more heterogeneous population. We discuss our control for this heterogeneity below.

Positive control dataset: MR-IMPACT study of depression.

To test the specificity of our findings, we analyzed a positive control dataset of a distinct psychiatric disorder, major depression. We obtained data from the MR-IMPACT study of depression, which comprises resting state scans from adolescent male (n=6) and female (n=18) controls, and adolescent male (n = 17) and female (n = 46) patients with moderate-to-severe major depressive disorder (43) but otherwise typical development (44).

Preprocessing

FMRI scanning parameters for the primary and replication dataset are provided in SI 2. A preprocessing pipeline using AFNI (45) and FSL (46) was applied to all scans. The pipeline included removal of the first five scans of each functional EPI series, skull-stripping, brain segmentation, non-linear registration to MNI space, and co-registration of anatomical images to re-aligned and slice-time corrected functional scans. Motion parameters, and mean signal from trimmed binary masks (partial volume estimates > 0.99) of cerebrospinal fluid and white matter, their derivatives and quadratic terms, were regressed out as confounds, resulting in a total of 32 regressors (47; 48); we did not perform global signal regression (49). Each participant's time-series were despiked, band-pass filtered in the range 0.01-0.1Hz,

denoised by removal of the 32 regressors (band-pass filtered in the same range), and smoothed with an 8-mm FWHM Gaussian kernel, all using the AFNI 3dBandpass command (https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dBandpass.html). Movement is an issue of high concern in analyses of functional connectivity (50–53), and we provide details of our pipeline and an analysis of the effect of motion on our results in SI 3.

The DMN was defined as 58 8mm-radius spherical regions of interest derived from a meta-analysis of fMRI studies (54) (SI 4). To remove weak and spurious correlations we analyzed binary networks obtained by thresholding the matrices, and preserving only the strongest 20% of connection weights for each participant.

Statistical analysis

We computed functional DMN intra-connectivity as the density of all binary intra-DMN edges minus a constant number of such edges expected in a random network (0.2 for a 20% density, see SI 5 for additional discussion). We defined functional connectivity using Pearson correlation and subtracted the constant to increase interpretability and decrease dependence of the measure on the chosen binarizing threshold. In the primary dataset, we tested for a difference between control females and 1) females with ASC, 2) sisters of subjects with ASC, 3) control males and 4) males with ASC, and between control males and males with ASC, using multiple regression controlling for effects of age and IQ. In the replication dataset we conducted the same tests (with the exception of the sibling contrast) but included an additional regressor of study site (thus correcting for age, IQ and study site). As the residuals of the test on the replication dataset failed a Shapiro-Wilk test for normality ($p = .03$), we employed robust regression for all analyses in this study, although results were similar with standard least squares regression. Robust regression, in comparison to standard

regression, is less affected by violations of normality and by the potential presence of outliers (55).

We quantified the final effect sizes by pooling all available data from the primary and replication datasets, and performing a multiple regression analysis, correcting for age, IQ and study site. We quantified differences in connectivity between groups as a percentage change of mean DMN intra-connectivity relative to a baseline of control males (SI 6). We tested the specificity of observed effects by repeating the multiple regression analysis on all data including the positive-control dataset, specifically testing for an effect of depression diagnosis. We repeated this test separately for both sexes. Finally, we explicitly investigated the effect of age, repeating the analysis on the pooled data of the primary and replication datasets stratified by age group: children (aged 6-11 years), adolescents (12-18 years) and adults (>18 years).

Robustness analyses

We conducted a number of robustness analyses, including the additional preprocessing step of scrubbing, exclusion of high-moving subjects, regressing out motion parameters, using a threshold-independent quantification of intra-network connectivity, and excluding three sites in the ABIDE dataset associated with previous studies of DMN connectivity (to exclude a possible circular argument). We also further explored the impact of motion. See SI 7 for full details on these analyses.

Behavioral analysis

Data was collected for all CFSA participants on performance on the "Reading the Mind in the Eyes" mentalizing task (38; 56). This task, performed during fMRI recording,

is a popular test of mentalizing and emotion recognition: presented with just a pair of eyes, participants were required to choose one of two words to describe the expression of the eyes and the congruent mental state. Although we do not have direct measures of systemizing or empathizing, this task is related to mentalizing as well as the empathizing construct. In a control, non-mentalizing condition, participants simply judged whether the eyes belonged to a male or female. Previously, we found performance on this mentalizing task to be related to diagnosis; such that subjects with autism performed worse than controls (38). Here we examined whether DMN intra-connectivity correlated with the percentage of incorrect responses in the mentalizing and the control condition beyond this diagnosis effect, by regressing out the effects of age, IQ and the six groups (ASC, sibling and control groups split by sex). We also performed this analysis separately for the two sexes and three groups.

Results

DMN hypoconnectivity was previously shown to characterize autism in male-only or heavily male-biased studies (22–30) and to appear as an endophenotype in male siblings (30). Here, controlling for heterogeneity in age and IQ, we found that DMN hypoconnectivity is likewise robustly present in females with ASC (primary dataset p = .001; replication dataset p = .009, Fig 1A,C) and also represents an endophenotype with unaffected female siblings of individuals with ASC placed between typically developing and autism participants, with significantly lower connectivity than the former (p = .035). The endophenotype analysis of the females complements our previous report of an endophenotype for the male-only subset of this dataset in a previous study (30). Furthermore, consistent with the hypothesized difference in autistic traits between typical males and females, DMN intra-connectivity was lower in control males than control females (primary dataset p = .036; replication dataset p = .002, Fig 1B,D).

We quantified the effect sizes for the four groups by pooling the primary and replication datasets (Fig 2A). The mean connectivity for control females was 27% higher than the mean value for control males, while the mean for males with ASC was 16% lower. Females with ASC were intermediate between males with ASC and control males, with mean 9% lower than the latter (not statistically significantly different from either group, $p > .1$).

Our pooled data covered a substantial age range from 6.5 to 58. Although DMN intra-connectivity appears variable across the lifespan (Fig. 2B), the contrasts we identified were present to some extent in all three age groups. We replicated all three testable comparisons, control females – females with ASC, control females – control males, control males – males with ASC, for children (effect size [p-value]: .08 [.009], .06 [.01], .04 [.02]) and adolescents (.06 [.02], .05 [.002], .03 [.004]). For adults, effect sizes were reduced and differences were trend-level or non-significant (.08 [.1], .02 [.5], .03 [.1]). See SI 8 for details of this analysis. We could not evaluate the endophenotype effect in the replication dataset as it did not contain siblings.

We found no significant default-mode connectivity effect in a positive control dataset of participants (n=63) diagnosed with a distinct psychiatric condition, major depressive disorder ($p > .1$; Fig. 2C). The effect remained absent when allowing for a sex by diagnosis interaction (SI 9).

Behavioral data are available from the CFSA sample only. The observed differences in DMN connectivity were significantly associated with performance on the "Reading the Mind in the Eyes" task (56), a task known to reveal mentalizing impairments in autism (Fig. 1E,F). A higher percentage of errors on this task was associated with lower DMN intra-connectivity in the whole sample of males and females with ASC, siblings and controls ($p = .001$), beyond the effects of diagnosis, age and IQ. The same effect was separately found in the female ($p = .034$) and male ($p = .016$) groups. A negative effect was also found when

196 analyzing each of the three (ASC, siblings, controls) groups separately, but only significantly
197 so for the ASC group ($p = .008$). See SI 10 for full results. Furthermore, there was no
198 relationship between DMN intra-connectivity and percentage errors in a control task of
199 gender-judgment ($p > .1$), and performance on neither task correlated with movement (SI 10).

200

201 ## Discussion

202 To our knowledge, this is the first direct investigation of functional connectivity in the
203 default mode network as neurobiological correlate of the sex-related spectrum of autistic
204 traits. We used two independently acquired datasets to test three specific predictions. Firstly,
205 we showed a robust and specific reduction in DMN intra-connectivity in females with ASC
206 and in unaffected female siblings of subjects with ASC, replicating previous results in males
207 (30). Secondly, we found that control females had an increased DMN intra-connectivity
208 compared to control males, and that people with ASC tended to have lower intra-connectivity
209 still. Thirdly and in line with this, we found that DMN intra-connectivity correlated with
210 performance in a behaviorally relevant mentalizing task that typically reveals deficits in
211 autism. These findings bring together two strands of research in the autism literature,
212 suggesting that abnormal DMN connectivity may underlie the spectrum of autistic traits that
213 extends into the general population. As reduced DMN intra-connectivity is consistently found
214 across males and females on the spectrum, and indeed differs in typically developing males
215 and females, we suggest it may be highly relevant to the autistic phenotype and autistic traits,
216 which appear to a greater extent in males.

217 Originally, the idea that ASC resembled an exaggerated manifestation of typical
218 sexual dimorphism was linked to the expression of systemizing and empathizing (7; 8),
219 psychological processes linked respectively to strengths and weaknesses in autism. To a

lesser extent than people with autism, typically developed males also tend to show strengths in the former and weaknesses in the latter; consequently, people with autism were said to show a form of the "extreme male brain" (7; 8). This theory has been extended in later years following the tentative relationship found between empathizing, systemizing, autistic traits and prenatal androgen exposure (10; 11), which is believed to permanently modify brain structure (57; 58). Although we cannot comment on this aspect of the male brain hypothesis, we add to this original theory by revealing that the most robust difference in functional connectivity in ASC is expressed on the same sex-related spectrum.

Many studies suggest that ASC in females is distinct at the level of brain and behavior from ASC in males (4; 59–66). Research continues to search for differences in genetics and for protective features that might set apart females with ASC (4; 67). There are nevertheless some brain and behavioral commonalities between males and females with ASC (4; 59; 60), and our study is to our knowledge the first functional connectivity investigation to report that reduced DMN intra-connectivity is shared across the sexes, with both males and females with ASC down the more 'male' end of the spectrum (Fig 2A). This finding, consistent with behavioral results (68), indicates that DMN connectivity may underlie some of the shared symptomatology of autism in males and females, and is consistent with the putative role of DMN in mentalizing and social cognition (14–18), known to be impaired in both males and females with ASC. Further evidence of this role was given by the correlation we observed between DMN intra-connectivity and performance on the mentalizing task, which relies on some of the same cognitive mechanisms as empathy (7), and lack of correlation with performance in a condition unrelated to mentalizing (gender-judgments). An EMB trend has recently been reported for females in whole brain connectivity during resting state (69), but future research should investigate whether these hypotheses apply to other aspects of brain and network structure and function,

The effects of age on functional connectivity in ASC has been a topic of recent interest (70). DMN intra-connectivity has been studied in children (23; 71), adolescents (22; 25; 28; 30) and adults (27) with ASC, and in wide-ranging groups spanning late adolescence to adulthood (24; 26; 29). On the whole, these lean towards hypoconnectivity with the exception of three studies which, on greater scrutiny, report hyperconnectivity between some individual nodes of the DMN (23; 25; 71) in contrast to the more expansive approach we take here. This may explain why, when studying the DMN as a larger whole, we saw reductions in DMN intra-connectivity that were common across age groups in ASC, appearing both in a tight age-matched group (aged 12-18 years) and a larger dataset with wide age range (between 6-58 years). Additional analyses on the latter revealed the effects to be significant for children (6-11 years) and adolescents (12-18 years) but only at trend level for adults (between 19-58 years). This seems consistent with the view that ASC are developmental conditions in which neurobiological differences may be at their most apparent in early life (72; 73). There has previously been suggestion, in ASC, of general hyperconnectivity in early life (70) and this was indeed seen between some nodes of the DMN (23; 71) but does not appear to be the case for DMN as a whole. Other studies, finding smaller and absent effects in adulthood and adolescence respectively, suggest that DMN connectivity develops on a markedly different trajectory in ASC (74; 75). This may be why there are null findings in some studies, if there is greater than average variability in the rate of development in people with ASC. As the current study employed a cross-sectional sample, longitudinal analysis in future research may help to clarify age-related changes in the DMN. Further research should also clarify whether effects of sex, as defined in the extreme male brain theory, are modulated by age.

It is important to note that our results apply to group differences, tendencies in large populations, and may not fully explain individual differences. For example, even though

people with ASC tend to fall on the (extreme) male end of the distribution (76), this is not true of every individual. This is well depicted in the distribution of the data in Figure 2: the different groups show clearly different profiles, all characterized by a large amount of within-group heterogeneity. In line with current views on insufficient emphasis on effect sizes (77–79), it is important to realize that heterogeneity can mask considerable effects. Indeed, we found an increase of 27% (respectively a decrease of 16%) in DMN connectivity for control females (respectively for ASD males) relative to control male participants. These findings suggest that DMN intra-connectivity represents an important risk factor in a multifactorial interplay underpinning the biological presentation of autism.

Motion can have a profound effect on estimates of functional connectivity (50–53). Non-trivial patterns of distance-dependent alterations of functional connectivity have been shown to be the result of motion artifacts, and many preprocessing strategies, such as the one we employed, have been used to correct for these. Motion is particularly problematic for studies of autism, as participants with ASC tend to move more in the scanner. However, our results were robust against a range of methods aimed to reduce motion artifacts (SI 7), including the additional step of scrubbing (SI 7.2). It is however noteworthy that a correlation with motion remained after these preprocessing steps. This remaining correlation is consistent with recent findings from Zeng et al (80). These authors presented evidence that DMN hypoconnectivity is a stable, biological trait that predisposes to movement, rather than an artifact caused by scanner movement: that individuals with lower connectivity in the DMN actually move more. We likewise found that DMN intra-connectivity correlated with motion in repeat scans for the same subjects, even after removing the effect of motion in the current scan (SI 3.3). Importantly, we additionally found a strong relationship between DMN intra-connectivity and performance on a mentalizing task, providing additional evidence for the claims of Zeng et al. that the correlation may represent biological, rather than artifactual

effects. Future research should further evaluate this hypothesis in more detail, investigating to which groups and under which circumstances it applies.

A notable feature of our study is the inclusion of a positive control group of patients with a distinct psychiatric disorder, major depression. This inclusion differentiates our work from the majority of neuroimaging autism literature, which does not include positive control subjects. In contrast to autism, there seems to be, on balance, greater evidence for DMN hyperconnectivity, rather than hypoconnectivity, in adults with major depression (81; 82). Another study, reporting more complex patterns of hyper- *and* hypoconnectivity within and between DMN and other brain regions, suggest that developmental changes with age may impact findings (83). While our adolescent depression data set was comparatively small, the absence of DMN hypoconnectivity in these data, at least, represents some evidence for specificity of our studied connectivity measure. More generally, the inclusion of positive controls in future studies represents an important goal towards more clinically relevant conclusions, and constitutes an important step towards translation of this and other neuroimaging phenotypes in ASC (84; 85).

In summary, our analyses suggest that the default-mode network shows a robust, heritable, specific and behaviorally relevant reduction across the autism spectrum. The analyses simultaneously reconcile two distinct strands of autism research, the extreme male brain theory of autism, and default-mode connectivity in autism, into a convergent and unified picture of biological abnormalities in autism.

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References

1. Chakrabarti S, Fombonne E (2005): Pervasive developmental disorders in preschool children: confirmation of high prevalence. *Am J Psychiatry*. 162: 1133–1141.

2. Giarelli E, Wiggins LD, Rice CE, Levy SE, Kirby RS, Pinto-Martin J, Mandell D (2010): Sex differences in the evaluation and diagnosis of autism spectrum disorders among children. *Disabil Health J*. 3: 107–16.

3. Begeer S, Mandell D, Wijnker-Holmes B, Venderbosch S, Rem D, Stekelenburg F, Koot HM (2013): Sex differences in the timing of identification among children and adults with autism spectrum disorders. *J Autism Dev Disord*. 43: 1151–6.

4. Lai M-C, Lombardo M V., Auyeung B, Chakrabarti B, Baron-Cohen S (2015): Sex/Gender Differences and Autism: Setting the Scene for Future Research. *J Am Acad Child Adolesc Psychiatry*. 54: 11–24.

5. Gould J, Ashton-Smith J (2011): Missed diagnosis or misdiagnosis? Girls and women on the autism spectrum. *Good Autism Pract*. 12: 34–41.

6. Asperger H (1944): Die “autistischen psychopathen” im kindesalter. *Eur Arch Psychiatry Clin Neurosci*. 117: 76–136.

7. Baron-Cohen S (2009): Autism: the empathizing-systemizing (E-S) theory. *Ann N Y Acad Sci*. 1156: 68–80.

8. Baron-Cohen (2002): The extreme male brain theory of autism. *Trends Cogn Sci*. 6: 248–254.

9. Robinson E, St Pourcain B, Anttila V, Kosmicki J, Bulik-Sullivan B, Grove J, *et al.* (2016): Genetic risk for autism spectrum disorders and neuropsychiatric variation in the general population. *Nat Genet*. . doi: 10.1038/ng.3529.

10. Auyeung B, Taylor K, Hackett G, Baron-Cohen S (2010): Foetal testosterone and autistic traits in 18 to 24-month-old children. *Mol Autism*. 1: 11.

11. Baron-Cohen S, Auyeung B, Nørgaard-Pedersen B, Hougaard DM, Abdallah MW, Melgaard L, *et al.* (2015): Elevated fetal steroidogenic activity in autism. *Mol Psychiatry*. 20: 369–376.

12. Geschwind DH, Levitt P (2007): Autism spectrum disorders: developmental disconnection syndromes. *Curr Opin Neurobiol*. 17.

13. Fox MD, Raichle ME (2007): Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci*. 8: 700–711.

14. Lombardo M V., Chakrabarti B, Bullmore ET, Sadek SA, Pasco G, Wheelwright SJ, *et al.* (2010): Atypical neural self-representation in autism. *Brain*. 133: 611–624.

15. Spreng RN, Mar RA, Kim ASN (2009): The common neural basis of autobiographical memory, prospection, navigation, theory of mind, and the default mode: a quantitative meta-analysis. *J Cogn Neurosci*. 21: 489–510.

16. Andrews-Hanna JR, Reidler JS, Sepulcre J, Poulin R, Buckner RL (2010): Functional-Anatomic Fractionation of the Brain’s Default Network. *Neuron*. 65: 550–562.

17. Mars RB, Neubert F-X, Noonan MP, Sallet J, Toni I, Rushworth MFS (2012): On the relationship between the "default mode network" and the "social brain." *Front Hum Neurosci*. 6.

18. Schilbach L, Eickhoff SB, Rotarska-Jagiela A, Fink GR, Vogeley K (2008): Minds at rest? Social cognition as the default mode of cognizing and its putative relationship to the "default system" of the brain. *Conscious Cogn*. 17: 457–467.

19. Kennedy DP, Redcay E, Courchesne E (2006): Failing to deactivate: resting functional abnormalities in autism. *Proc Natl Acad Sci U S A*. 103: 8275–8280.

20. Iacoboni M (2006): Failure to deactivate in autism: the co-constitution of self and other. *Trends Cogn Sci*. 10.

21. Spencer MD, Chura LR, Holt RJ, Suckling J, Calder AJ, Bullmore ET, Baron-cohen S (2012): Failure to deactivate the default mode network indicates a possible endophenotype of autism. *Mol Autism*. 3: 1–9.

22. Assaf M, Jagannathan K, Calhoun VD, Miller L, Stevens MC, Sahl R, *et al.* (2010): Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. *Neuroimage*. 53: 247–56.

23. Lynch CJ, Uddin LQ, Supekar K, Khouzam A, Phillips J, Menon V (2013): Default mode network in childhood autism: Posteromedial cortex heterogeneity and relationship with social deficits. *Biol Psychiatry*. 74: 212–219.

24. 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. *Soc Cogn Affect Neurosci*. 8: 694–701.

25. Redcay E, Moran JM, Mavros PL, Tager-Flusberg H, Gabrieli JDE, Whitfield-Gabrieli S (2013): Intrinsic functional network organization in high-functioning adolescents with autism spectrum disorder. *Front Hum Neurosci*. 7: 573.

26. Cherkassky VL, Kana RK, Keller TA, Just MA (2006): Functional connectivity in a baseline resting-state network in autism. *Neuroreport*. 17: 1687–1690.

27. Monk CS, Peltier SJ, Wiggins JL, Weng SJ, Carrasco M, Risi S, Lord C (2009): Abnormalities of intrinsic functional connectivity in autism spectrum disorders,. *Neuroimage*. 47: 764–772.

28. Weng S-J, Wiggins JL, Peltier SJ, Carrasco M, Risi S, Lord C, Monk CS (2010): Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. *Brain Res*. 1313: 202–14.

29. Kennedy DP, Courchesne E (2008): The intrinsic functional organization of the brain is altered in autism. *Neuroimage*. 39: 1877–85.

30. Moseley RL, Ypma RJF, Holt RJ, Floris D, Chura LR, Spencer MD, *et al.* (2015): Whole-brain functional hypoconnectivity as an endophenotype of autism in adolescents. *NeuroImage Clin*. 9: 140–152.

31. Glahn DC, Knowles EEM, McKay DR, Sprooten E, Raventós H, Blangero J, *et al.* (2014): Arguments for the sake of endophenotypes: Examining common misconceptions about the use of endophenotypes in psychiatric genetics. *Am J Med Genet Part B Neuropsychiatr Genet*. 165: 122–130.

32. Biswal BB, Mennes M, Zuo X-N, Gohel S, Kelly C, Smith SM, *et al.* (2010): Toward discovery science of human brain function. *Proc Natl Acad Sci U S A*. 107: 4734–9.

33. Allen E a, Erhardt EB, Damaraju E, Gruner W, Segall JM, Silva RF, *et al.* (2011): A baseline for the multivariate comparison of resting-state networks. *Front Syst Neurosci*. 5: 2.

34. Weissman-Fogel I, Moayedi M, Taylor KS, Pope G, Davis KD (2010): Cognitive and default-mode resting state networks: Do male and female brains "rest" differently? *Hum Brain Mapp*. 31: 1713–1726.

35. Spencer MD, Holt RJ, Chura LR, Calder AJ, Suckling J, Bullmore ET, Baron-Cohen S (2012): Atypical activation during the Embedded Figures Task as a functional magnetic resonance imaging endophenotype of autism. *Brain*. 135: 3469–3480.

36. Spencer MD, Holt RJ, Chura LR, Suckling J, Calder a J, Bullmore ET, Baron-Cohen S (2011): A novel functional brain imaging endophenotype of autism: the neural response to facial expression of emotion. *Transl Psychiatry*. 1: e19.

37. Floris DL, Chura LR, Holt RJ, Suckling J, Bullmore ET, Baron-Cohen S, Spencer MD (2013): Psychological correlates of handedness and corpus callosum asymmetry in autism: the left hemisphere dysfunction theory revisited. *J Autism Dev Disord*. 43: 1758–72.

38. Holt RJ, Chura LR, Lai M-C, Suckling J, von dem Hagen E, Calder a J, *et al.* (2014): "Reading the Mind in the Eyes": an fMRI study of adolescents with autism and their siblings. *Psychol Med*. 44: 3215–27.

39. Lisiecka DM, Holt R, Tait R, Ford M, Lai M-C, Chura LR, *et al.* (2015): Developmental white matter microstructure in autism phenotype and corresponding endophenotype during adolescence. *Transl Psychiatry*. 5: e529.

40. 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. *J Autism Dev Disord*. 30: 205–223.

41. Le Couteur A, Lord C, Rutter M (2003): *The autism diagnostic interview-revised (ADI-R)*. Los Angeles, CA: Western Psychological Services.

42. Di Martino a, Yan C-G, Li Q, Denio E, Castellanos FX, Alaerts K, *et al.* (2014): The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. *Mol Psychiatry*. 19: 659–67.

43. American Psychiatric Association (2000): *Diagnostic and Statistical Manual of Mental Disorders*, Fourth. Washington, DC: American Psychiatric Association.

44. Hagan CC, Graham JM, Widmer B, Holt RJ, Ooi C, van Nieuwenhuizen AO, *et al.* (2013): Magnetic resonance imaging of a randomized controlled trial investigating predictors of recovery following psychological treatment in adolescents with moderate to severe unipolar depression: study protocol for Magnetic Resonance-Improving Mood with Psycho. *BMC Psychiatry*. 13: 247.

45. Cox RW (1996): AFNI: Software for Analysis and Visualization of Functional Magnetic Resonance Neuroimages. *Comput Biomed Res*. 29: 162–173.

46. Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM (2012): FSL. *Neuroimage*. 62.

47. Satterthwaite TD, Elliott MA, Gerraty RT, Ruparel K, Loughead J, Calkins ME, *et al.* (2013): An improved framework for confound regression and fi ltering for control of motion artifact in the preprocessing of resting-state functional connectivity data. *Neuroimage*. 64: 240–256.

48. Patel AX, Kundu P, Rubinov M, Jones PS, Vértes PE, Ersche KD, *et al.* (2014): A wavelet method for modeling and despiking motion artifacts from resting-state fMRI time series. *Neuroimage*. 95: 287–304.

49. Gotts SJ, Saad ZS, Jo HJ, Wallace GL, Cox RW, Martin A (2013): The perils of global signal regression for group comparisons: a case study of Autism Spectrum Disorders. *Front Hum Neurosci*. 7: 356.

50. Satterthwaite TD, Wolf DH, Loughead J, Ruparel K, Elliott MA, Hakonarson H, *et al.* (2012): Impact of in-scanner head motion on multiple measures of functional connectivity: Relevance for studies of neurodevelopment in youth. *Neuroimage*. 60: 623–632.

51. van Dijk KRA, Sabuncu MR, Buckner RL (2012): The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage*. 59: 431–438.

52. 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: 320–41.

53. Power JD, Schlaggar BL, Petersen SE (2015): Recent progress and outstanding issues in motion correction in resting state fMRI. *Neuroimage*. 105: 536–551.

54. Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church J a, *et al.* (2011): Functional network organization of the human brain. *Neuron*. 72: 665–78.

55. Huber P (1981): *Robust statistics*. New York, NY: Wiley.

56. Baron-Cohen S, Wheelwright S, Hill J, Raste Y, Plumb I (2001): The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry*. 42: 241–251.

57. Arnold AP (2009): The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Horm Behav*. 55: 570–578.

58. Bao A-M, Swaab DF (2011): Sexual differentiation of the human brain: relation to gender identity, sexual orientation and neuropsychiatric disorders. *Front Neuroendocrinol*. 32: 214–226.

59. Lai MC, Lombardo M V., Suckling J, Ruigrok AN V, Chakrabarti B, Ecker C, *et al.* (2013): Biological sex affects the neurobiology of autism. *Brain*. 136: 2799–2815.

60. Lai MC, Lombardo M V., Ruigrok AN V, Chakrabarti B, Wheelwright SJ, Auyeung B, *et al.* (2012): Cognition in Males and Females with Autism: Similarities and Differences. *PLoS One*. 7. doi: 10.1371/journal.pone.0047198.

61. Dworzynski K, Ronald A, Bolton P, Happé F (2012): How different are girls and boys

above and below the diagnostic threshold for autism spectrum disorders? *J Am Acad Child Adolesc Psychiatry*. 51: 788–97.

62. Frazier TW, Georgiades S, Bishop SL, Hardan AY (2014): Behavioral and cognitive characteristics of females and males with autism in the Simons Simplex Collection. *J Am Acad Child Adolesc Psychiatry*. 53: 329–340.

63. Hiller RM, Young RL, Weber N (2014): Sex differences in autism spectrum disorder based on DSM-5 criteria: evidence from clinician and teacher reporting. *J Abnorm Child Psychol*. 42: 1381–93.

64. Kirkovski M, Enticott PG, Fitzgerald PB (2013): A review of the role of female gender in autism spectrum disorders. *J Autism Dev Disord*. 43: 2584–603.

65. Rivet TT, Matson JL (2011): Review of gender differences in core symptomatology in autism spectrum disorders. *Res Autism Spectr Disord*. 5: 957–976.

66. Bloss CS, Courchesne E (2007): MRI neuroanatomy in young girls with autism: a preliminary study. *J Am Acad Child Adolesc Psychiatry*. 46: 515–523.

67. Robinson EB, Lichtenstein P, Anckarsäter H, Happé F, Ronald A (2013): Examining and interpreting the female protective effect against autistic behavior. *Proc Natl Acad Sci U S A*. 110: 5258–62.

68. Baron-Cohen S, Bowen DC, Holt RJ, Allison C, Auyeung B, Lombardo M V., *et al.* (2015): The "Reading the Mind in the Eyes" Test: Complete Absence of Typical Sex Difference in ~400 Men and Women with Autism. *PLoS One*. 10: e0136521.

69. Alaerts K, Swinnen S, Wenderoth N (2016): Sex differences in autism: A resting-state fMRI investigation of functional brain connectivity in males and females. *Soc Cogn Affect Neurosci*. . doi: 10.1093/scan/nsw027.

70. Nomi JS, Uddin LQ (2015): Developmental changes in large-scale network connectivity in autism. *NeuroImage Clin*. 7: 732–741.

71. Uddin LQ, Supekar K, Lynch CJ, Khouzam A, Phillips J, Feinstein C, *et al.* (2013): Salience network-based classification and prediction of symptom severity in children with autism. *JAMA psychiatry*. 70: 869–79.

72. Dajani D, Uddin L (2016): Local brain connectivity across development in autism spectrum disorder: A cross-sectional investigation. *Autism Res*. 9: 43–54.

73. Hazlett HC, Poe MD, Gerig G, Styner M, Chappell C, Smith RG, *et al.* (2011): Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. *Arch Gen Psychiatry*. 68: 467–476.

74. Burrows CA, Laird AR, Uddin LQ (2016): Functional connectivity of brain regions for self- and other-evaluation in children, adolescents and adults with autism. *Dev Sci*. 1–17.

75. Washington SD, Gordon EM, Brar J, Warburton S, Sawyer AT, Wolfe A, *et al.* (2014): Dysmaturation of the default mode network in autism. *Hum Brain Mapp*. 35: 1284–1296.

76. Baron-Cohen S (2005): Testing the extreme male brain (EMB) theory of autism: let the data speak for themselves. *Cogn Neuropsychiatry*. 10: 77–81.

77. Greenwald AG, Gonzalez R, Harris RJ, Guthrie D (1996): Effect sizes and p values:

What should be reported and what should be replicated? *Psychophysiology*. 33: 175–183.

78. Thompson B (2007): Effect sizes, confidence intervals, and confidence intervals for effect sizes. *Psychol Sch*. 44: 423–432.

79. Halsey LG, Curran-Everett D, Vowler SL, Drummond GB (2015): The fickle P value generates irreproducible results. *Nat Methods*. 12: 179–185.

80. Zeng L-L, Wang D, Fox MD, Sabuncu M, Hu D, Ge M, *et al.* (2014): Neurobiological basis of head motion in brain imaging. *Proc Natl Acad Sci U S A*. 111: 6058–62.

81. Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H, *et al.* (2007): Resting-State Functional Connectivity in Major Depression: Abnormally Increased Contributions from Subgenual Cingulate Cortex and Thalamus. *Biol Psychiatry*. 62: 429–437.

82. Whitfield-Gabrieli S, Ford JM (2012): Default mode network activity and connectivity in psychopathology. *Annu Rev Clin Psychol*. 8: 49–76.

83. Connolly CG, Wu J, Ho TC, Hoeft F, Wolkowitz O, Eisendrath S, *et al.* (2013): Resting-state functional connectivity of subgenual anterior cingulate cortex in depressed adolescents. *Biol Psychiatry*. 74: 898–907.

84. Kapur S, Phillips a G, Insel TR (2012): Why has it taken so long for biological psychiatry to develop clinical tests and what to do about it? *Mol Psychiatry*. 17: 1174–9.

85. Rubinov M, Bullmore E (2013): Fledgling pathoconnectomics of psychiatric disorders. *Trends Cogn Sci*. 17.

Figure Legends

Figure 1. Predictions for default-mode connectivity in a sex-related autistic trait spectrum. Group differences in DMN intra-connectivity, for (a) 66 and (b) 75 participants from the primary dataset (20 female controls present twice) and (c) 144 and (d) 925 participants from the replication dataset (89 female controls present twice). (e, f) Relationships between DMN intra-connectivity and performance on a mentalizing task, Spearman's ρ is given. All data are shown in (e), and split by sex in (f). Box-plots give quartiles, asterisks reflect significant differences: *p<.05, **p<.01, ***p<.001.

Figure 2. DMN intra-connectivity distributions derived from pooling the primary and replication datasets. The effects of age, IQ and site have been regressed out. (a) The distribution of DMN intra-connectivity for the four groups (top) and the four groups and positive control participants with major depressive disorder (bottom). The latter category does not differ from the control subjects. The panel illustrates both a clear difference between the mean values of the groups and large within-group heterogeneities. (b) The effect of age on these values. The lines for females are more volatile due to lower numbers, especially for adult ages.