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Psychotropic medication use in autism spectrum disorders may affect functional brain connectivity

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Abstract

Background—Prescription of psychotropic medications is common in autism spectrum disorders (ASDs), either off-label or to treat comorbid conditions such as ADHD or depression. Psychotropic medications are intended to alter brain function. Yet, studies investigating the functional brain organization in ASDs rarely take medication usage into account. This could explain some of the inconsistent findings of atypical brain network connectivity reported in the autism literature.

Methods—The current study tested whether functional connectivity patterns, as assessed with functional magnetic resonance imaging (fMRI), differed in a cohort of 49 children and adolescents with ASDs based on psychotropic medication status, and in comparison with 50 matched typically developing (TD) participants. Twenty-five participants in the ASD group (51%) reported current psychotropic medication usage, including stimulants, antidepressants, antipsychotics, and anxiolytics. Age, IQ, head motion, and ASD symptom severity did not differ between groups. Whole-brain functional connectivity between 132 regions of interest was assessed.

Results—Different functional connectivity patterns were identified in the ASD group taking psychotropic medications (ASD-on), as compared to the TD group and the ASD subgroup not using psychotropic medications (ASD-none). The ASD-on group showed distinct underconnectivity between the cerebellum and basal ganglia but cortico-cortical overconnectivity compared to the TD group. Cortical underconnectivity relative to the TD pattern, on the other hand, was pronounced in the ASD-none group.

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Conclusions—These results suggest that psychotropic medications may affect functional connectivity, and that medication status should be taken into consideration when studying brain function in autism.

Keywords

Autism spectrum disorders; functional connectivity; fMRI; psychotropic medication; repetitive behaviors; comorbidity

Autism spectrum disorders (ASDs) are life-long neurodevelopmental disorders characterized by repetitive behaviors, and social and communicative deficits such as difficulties recognizing others' emotions or intentions. Currently there are no pharmacological treatments specifically developed to target symptoms of ASDs, but the use of psychostimulants, antidepressants, anxiolytics and even antipsychotics has become common, either off-label to alleviate symptoms or to treat comorbid conditions such as ADHD and anxiety (1–6). A recent study of 33,565 commercially insured children with ASDs in the US for example found that 64% were prescribed psychotropic medication and 35% had evidence of psychotropic polypharmacy (7). Rates of prescription drug use for children with ASDs have risen in recent years (8), with marked increases in antipsychotic use (9). In adults these rates are similar (10) or even higher (11). These medications are designed to alter brain function, and consequently emotions and behavior, by acting on neurotransmitters and associated receptors (12).

Numerous reviews and meta-analyses of neuroimaging studies in both children and adults with schizophrenia, bipolar disorder, depression, and ADHD have shown potential effects of psychotropic medications on brain function (13–19). In adults with bipolar disorder, for example, psychotropic medication (including mood stabilizers, antipsychotics, antidepressants, and anxiolytics) is associated with increased activity in the dorsolateral prefrontal cortex, and increases in gray matter in the anterior cingulate gyrus, amygdala, and hippocampus, overall indicating a normalizing effect of medication on brain function and structure (14, 16). In adults with schizophrenia, antipsychotic use is associated with increased functional connectivity between the medial frontal cortex (MFC) and left nucleus accumbens, but decreased functional connectivity between MFC and right hippocampus (20), and normalizing effects on the BOLD signal (13). In healthy adults, acute effects of stimulant use (i.e., methylphenidate) include enhanced functional connectivity of somatomotor cortex with thalamus and striatum (21), while use of selective serotonin reuptake inhibitors (SSRIs) is linked to decreased default mode network connectivity and increased connectivity between PCC and auditory network (22). In children with ADHD, methylphenidate use is associated with enhanced activation in bilateral inferior frontal cortex and insula (17) and normalized resting-state connectivity (23).

In light of these findings, it is remarkable how few functional magnetic resonance imaging (fMRI) studies in ASDs have taken psychotropic medication status into account. Among the few exceptions are Just et al. (24) and Weng et al. (25) who reported no differences in brain activation between children with ASDs taking medications and those who did not. Kennedy et al. (26) found a slightly different pattern of results when excluding participants with

ASDs on psychotropic medication. However, these findings were limited by small samples (9/18, 10/16 and 4/12 participants with ASDs on medication, respectively). While altered functional brain network connectivity has been found in numerous studies of ASDs (27–32), there is little consensus about ways in which connectivity is disrupted, with diverse patterns of under- and overconnectivity compared to TD controls observed across studies. Some of these inconsistencies have been attributed to differences in methodology (33) and demographics such as age (34). In the current study, we assessed whether the use of psychotropic medication could also contribute to the variability in patterns of functional connectivity in a large cohort of children and adolescents with ASDs.

Methods and Materials

Participants

Data from 49 children and adolescents with ASDs, ages of 8–17 years, and 50 TD participants matched on age, gender, handedness, non-verbal IQ and in-scanner head motion were analyzed for this study (see Table 1). ASD diagnoses were confirmed with the Autism Diagnostic Observation Schedule, 2nd Edition (ADOS-2) (35) and the Autism Diagnostic Interview Revised (ADI-R) (36), with expert clinical decision based on DSM-5 criteria (American Psychiatric Association, 2013). Participants with comorbid ASD-related medical conditions (e.g., Fragile-X syndrome, tuberous sclerosis, epilepsy), or other neurological conditions (e.g., Tourette syndrome) were excluded. However, participants with comorbid ADHD, OCD, or anxiety disorders were not excluded, because of the high prevalence of such conditions among children and adolescents with ASDs (37). Typically developing children were also screened for any personal or family history of neurological, psychiatric, or developmental disorders. All participants were safety-screened for MRI contraindications (e.g., claustrophobia, ferrous material in body). Informed assent and consent were acquired from all participants and their caregivers. The study protocol was approved by the institutional review boards of the University of California San Diego and San Diego State University.

Participants were chosen from a larger cohort (ASDs: n=88, TD: n=64) based on availability of both high-quality resting state fMRI data and information on psychotropic medication usage. 10 participants (9 ASD, 1 TD) did not have sufficient information about current medication status, and three ASD participants did not complete the MRI session. Excessive motion during resting state fMRI scan led to the exclusion of 29 participants (23 ASD, 6 TD, with >20% of imaging data corrupted by >0.5 mm motion). Data from additional 11 participants were excluded due to excessive drowsiness during the scan (1 TD), MRI image artifacts (1 ASD, 4 TD), or incidental finding on MRI (3 ASD, 2 TD), leaving 49 children with ASDs and 50 TD youths in the final sample.

Participants with ASDs were subdivided into two groups based on current psychotropic medication use, as reported by their caregivers. Medication details are provided in Table 2. Of the 49 ASD participants, 25 (51%) were using psychotropic medications (ASD-on), including stimulants (n=14), mood stabilizers (n=12), SSRI/antidepressants (n=16), and anxiolytics/other (n=11). Note, that 18 participants reported use of more than one psychotropic medication. 15 participants (60%) in the ASD-on group reported at least one

additional diagnosis (Table 3). Reported comorbid diagnoses included ADHD (n=10), depression (n=5), and anxiety (n=8), with 20% (n=5) reporting two or more comorbid diagnoses (in addition to ASD). The remaining 24 (49%) ASD participants were categorized as the ASD-none group. All ASD-none and TD participants reported no psychotropic medication use at the time of the scan or in the past. There were no significant differences in age, non-verbal IQ, and in-scanner head motion between the TD and two ASD groups (Table 1).

Diagnostic, Neuropsychological and Cognitive Assessments

ADOS-2 (35) and ADI-R (36) are standardized, semi-structured assessments of ASD symptomatology and were administered to all ASD participants (ADOS-2) and their caregivers (ADI-R) to confirm diagnosis. The Wechsler Abbreviated Scale of Intelligence, 2nd edition (WASI-II) (38) was administered to assess overall cognitive functioning. The Behavior Rating Inventory of Executive Function (BRIEF) (39), a caregiver questionnaire, was used to obtain a standardized measure of executive functioning in daily life across multiple domains. Differences in diagnostic, neuropsychological and cognitive assessments were examined using independent sample t-tests when comparing the ASD-on and ASD-none groups, and ANOVAs when comparing the two ASD and the TD groups.

Magnetic Resonance Imaging Data Acquisition

A mock scan session prior to the MRI scan acclimated the participants to the MR environment and allowed them to practice staying still.

Imaging data were acquired on a GE 3T Discovery MR750 scanner using an 8-channel head coil at the University of California San Diego Center for Functional MRI (CFMRI). A standard FSPGR T1-weighted sequence was used to acquire high-resolution structural images (172 slices; repetition time [TR] = 8.136 ms; echo time [TE] = 3.172 ms; flip angle = 8°; field of view [FOV] = 25.6 mm; matrix = 256 × 256; resolution = 1 mm³). Functional images were obtained using a single-shot gradient-recalled, echo-planar image pulse sequence. During the resting state scan, 180 whole-brain volumes were acquired (TR = 2000 ms; TE = 30 ms; slice thickness = 3.4 mm; flip angle = 90°; FOV = 22.0 mm; matrix = 64 × 64; in-plane resolution = 3.4 mm²) over the duration of 6 minutes. Participants were instructed to “Keep your eyes on the cross. Let your mind wander, relax, but please stay as still as you can. Do not to fall asleep.” Participants’ adherence to the instructions to remain awake, with eyes open, was monitored with an MR-compatible video camera.

Functional Magnetic Resonance Imaging Data Preprocessing

Functional MRI data were preprocessed and analyzed in Matlab 2015b (Mathworks Inc., Natick, MA, USA) using SPM 12 (Wellcome Trust Centre for Neuroimaging, University College London, UK) and the CONN toolbox(40) v15. Images were unwarped, slice-timing corrected, and motion corrected using rigid-body realignment as implemented in SPM. The root-mean-squared-difference (RMSD) was calculated from the 6 motion parameters (3 translational and 3 rotational) as an head motion estimate for each participant. The Artifact Detection Toolbox (ART(41): http://www.nitrc.org/projects/artifact_detect/) was used to detect motion outliers in the functional image timeseries with frame-wise displacement >0.5

mm or changes in signal intensity greater than three standard deviations. No participant included in this analysis had more than 20% of time points exceeding 0.5 mm frame-wise displacement. The number of outlier time points did not differ between the three groups ($F(2,96)=.016$, $p=.98$). Functional images were co-registered to the T1 structural image using non-linear transformation. The structural image was tissue segmented to yield white matter and CSF masks for each individual subject, and the structural and functional images were normalized to the MNI template. Functional images were bandpass filtered using a temporal filter of 0.008 to 0.08 Hz, and spatially smoothed with a 6 mm FWHM Gaussian smoothing kernel. To minimize the effect of head motion and reduce noise in the fMRI timeseries, motion outliers as detected by the ART toolbox were censored, and the 6 motion parameters derived from rigid-body realignment and their derivatives, as well as the first component timeseries derived from the CSF and white matter masks using aCompCor (42) and corresponding derivatives, were regressed out from the signal.

Functional Connectivity Analysis

Functional connectivity between 132 regions of interest (ROIs) obtained from the FSL Harvard-Oxford cortical and subcortical atlas (43) and the AAL cerebellar regions (44) (see default atlas in CONN toolbox) was assessed, providing whole-brain coverage. These well established structural atlases have been used in previous exploratory studies of functional connectivity differences in ASDs (e.g. (45)). Additionally, in the absence of functional parcellations derived from children or adolescents, structural atlases were considered preferable for this age group. Average timecourses were extracted from each ROI and functional connectivity between all ROI pairs was calculated using bivariate Pearson correlations standardized with a Fisher z-transform. A second-level general linear model tested for differences in connectivity between the ASD-on, ASD-none, and TD groups. Results for connectivity between pairs of ROIs are reported at a threshold of $p<.05$, FDR-corrected for multiple comparisons. Uncorrected results (thresholded at $p<.05$) are also shown to illustrate the pattern of overall differences in functional connectivity across the whole brain.

Results

Cognitive functioning and ASD symptoms

The ASD-on group did not differ from the ASD-none group or from the TD group on age ($F(2,96)=.43$, $p=.65$), in-scanner head motion (RMSD: $F(2,96)=.32$, $p=.73$; number of censored time points: $F(2,96)=.04$, $p=.96$), non-verbal ($F(2,96)=.10$, $p=.90$) or full-scale IQ ($F(2,96)=.57$, $p=.57$), see Table 1. There were no differences in current ASD symptomatology as measured by ADOS-2 Social Affect, Total and Severity subscales, between the ASD-on and ASD-none groups (all $ps > .20$, see Table 1 and Figure 1). However, the ASD-on group had slightly higher scores on the ADOS-2 Restricted and Repetitive Behavior (RRB) assessment ($t(39)=-1.99$, $p=.05$). There were no significant differences in early-life symptoms (symptoms present at 4–5 years of age) as assessed with ADI-R (all $ps > .20$, see Table 1 and Figure 1). Finally, comparison of the BRIEF subscales revealed more impaired current daily life executive functioning in the ASD-on group, with significantly higher scores on the BRIEF Behavioral Regulation Index (BRI) subscale

($t(41) = -3.01, p = .004$) and on the BRIEF Global Executive Component (GEC, $t(41) = -2.64, p = .012$), and a similar but non-significant pattern on the BRIEF Metacognition Index (MI; $t(41) = -1.83, p = .07$).

Functional connectivity

Whole-brain functional connectivity in the ASD-on group, compared to the TD group, was characterized by predominant cortico-cortical overconnectivity, but underconnectivity between cerebellum and basal ganglia. Pairwise ROI connectivity is shown at the uncorrected $p < .05$ level in Figure 2A, and FDR-corrected for multiple comparisons ($p < .05$) in Figure 2B. The ASD-none group on the other hand, showed predominant cortico-cortical underconnectivity compared to the TD group. Direct comparison between the ASD-on and ASD-none groups corroborated this pattern, with the ASD-on group showing cortico-cortical overconnectivity and cerebellum-basal ganglia underconnectivity (Figure 2A), although this was not significant with FDR correction.

In order to assess the extent to which this pattern was driven by psychotropic medication usage rather than group differences in core autism symptomatology, the ASD group was median-split on the ADOS-2 RRB scores ($n = 41$, median = 3) as this was the only subscale of the ADOS-2 for which there was a significant difference between the ASD-on ($m = 3.5$) and ASD-none ($m = 2.5$) groups. ASD participants with a score lower than the median constituted the ASD-low severity group ($n = 16$, $m = 1.25$, $SD = 0.77$), and participants with a score higher than the median the ASD-high severity group ($n = 18$, $m = 4.61$, $SD = 0.85$). Participants with a median score were not included in this analysis ($n = 7$). Unlike the pattern of findings when comparing ASD groups based on medication usage, the ASD-high group showed predominant cortico-cortical underconnectivity compared to the TD and ASD-low group (see Figure S1). Next, the analysis comparing the ASD-on and ASD-none group was repeated controlling for ADOS-2 RRB scores. The results were very similar, and are shown in Figure S2. This suggests that the connectivity differences seen between the ASD-on and ASD-none groups were not solely driven by core ASD symptom severity.

To assess whether the patterns of connectivity found in our in-house sample would replicate in an independent dataset with similar demographics, we compared our findings in a subset of participants who were prescribed stimulants ($n = 14$, including in combination with other psychotropic medication) with an age-matched group of participants taking stimulants at the time of the MRI scan from one ABIDE-I sample (UCLA-I, see Supplementary Methods). Despite small sample sizes and polypharmacy with a variety of secondary psychotropic medications being common, there was clear overlap in how functional connectivity differed between ASD-on, ASD-none and TD groups. As in our in-house sample, the UCLA participants taking stimulants showed subcortical underconnectivity compared to the TD and ASD-none groups, while the ASD-none group showed cortico-cortical underconnectivity compared to the TD group (see Figures S3 and S4).

Discussion

Given the high rates of psychotropic medication use in ASDs (1–6), the intended effects these medications have on the brain, and the changes in brain function as detected with

fMRI in other neuropsychiatric and neurodevelopmental disorders (18), it is surprising that many fMRI studies on ASDs have not taken medication status into account. In this study, we therefore tested for differences in functional connectivity between children and adolescents with ASD who were on medication and those who were not. Almost half of our cohort of children and adolescents with ASD were current users of psychotropic medications. In comparisons with a matched TD group and against each other, ASD-on and ASD-none groups showed overall different patterns of functional connectivity. The current findings suggest that psychotropic medication status can influence the results of fMRI studies comparing ASD and TD groups.

Behavioral differences

Previous studies have frequently found improvements of symptom severity with the usage of psychotropic medications in other neuropsychiatric and neurodevelopmental disorders (e.g., in ADHD, bipolar disorder, schizophrenia; see ref. (18) for review). There was no corresponding finding in our cohort of children and adolescents with ASDs taking psychotropic medications at the time of assessment. We found that the ASD-on group had significantly higher ADOS-2 scores for restricted and repetitive behaviors (RRB), and also increased executive functioning difficulties in daily life, as measured by the BRIEF. Caregivers did not report less severe early-life autism symptoms on the ADI-R, however. Since in our study it was not possible to directly compare the same cohort of children in both on-medication and off-medication conditions, these findings need to be taken with caution, however.

Importantly for the interpretation of the functional connectivity results (46), we found that the ASD subgroup on psychotropic medication did not differ in head motion during fMRI scanning from the ASD subgroup without current psychotropic medication use. RMSD and the number of time points censored also did not correlate with the BRIEF and ADOS-2 RRB scores that were significantly different in the ASD-on compared to ASD-none group. Interestingly, a recent study by Torres et al. (47) showed differences in the stochastic nature of motion fluctuations during fMRI scans with psychotropic medication usage in the Autism Brain Imaging Data Exchange cohort (ABIDE; N=1048 including ASD and TD participants, aged 5–60 years). Based on their findings, the authors suggest that psychotropic medications may be related to increased neuromotor symptomatology in individuals with ASDs. The higher ADOS-2 RRB scores and increased executive functioning difficulties found in our ASD-on group may reflect a similar relationship in our cohort.

Alterations of functional connectivity based on medication status

Overall, differences in functional connectivity patterns compared to the TD group varied in the ASD-on and ASD-none groups. Strikingly, not a single effect surviving FDR correction was found to be shared between the two ASD subgroups. Visual inspection of functional connectivity effects relative to the TD group suggests overall more cortico-cortical overconnectivity in the ASD-on group, and predominantly underconnectivity in the ASD-none group. These results may relate to findings from a small sample of 10 adults with ASDs indicating that beta-blockers (antihypertensive medications, commonly used in psychiatry to treat anxiety) increase functional connectivity between inferior frontal,

fusiform, parietal and middle temporal cortex during a verbal decision-making task (48). This is the only study we are aware of that has explicitly assessed the effect of medication on functional connectivity in autism. In ADHD, stimulant use has also been shown to strengthen functional connectivity in frontoparietal cortices (49).

In view of the ongoing debate about divergent functional connectivity findings in ASDs and potential methodological (33,50) or demographic explanations(34), the differential patterns of connectivity observed in the ASD-on and ASD-none groups are of interest because they suggest that medication status may be one additional factor contributing to functional connectivity variability. Grouping participants by ASD diagnosis for comparison with TD peers without taking medication status into account might also increase within-group variability, thus reducing the probability of detecting significant effects at the group level; for example if certain medications drive connectivity in opposite directions to what is observed in medication-naïve children with ASDs, or if psychotropic medications normalize brain function to be more similar to the TD group, as has been observed for medication use in other disorders (13, 14, 16, 23). Importantly, psychotropic medication use in ASDs is linked to an increased rate of comorbidities, and in our study was also related to increased repetitive behaviors and reduced executive function as measured by the BRIEF. It is not possible in the current study to discern whether differences in functional connectivity seen in the ASD-on and ASD-none group were due to psychotropic medication use or to underlying differences in symptomatology or comorbidity.

It should also be noted, that while most functional connectivity studies of ASDs to date have not disclosed medication status, some studies detected overconnectivity relative to TD participants in ASD cohorts with only a minority of participants on medications. For two examples from our own group, Fishman et al.(51) found overconnectivity between mirror neuron and theory of mind networks in 25 adolescents with ASD, 10 of whom (40%) were on diverse psychotropic medications. Khan et al. (52) detected robust overconnectivity between multiple cerebral cortical and cerebellar regions in a sample of 33 children and adolescents with ASDs, only 9 of whom were taking psychotropic medications. Any simple conclusion, according to which the brain with ASD would be underconnected off medication, but overconnected on medication, is therefore implausible. The pattern of differences that may be affected by medication status likely depends on the class, combination, treatment duration, and dosage of medications taken. However, our findings do suggest that medication status could be one factor (in addition to the mentioned methodological and demographic factors) that may contribute to differences in functional connectivity findings across studies.

Underconnectivity between cerebellum and basal ganglia in ASD group on medication

A very distinct pattern of underconnectivity between cerebellar ROIs (lobule 9, including vermis) and basal ganglia (right nucleus accumbens, bilateral putamen) was detected for the ASD-on group in comparison with the TD group. Differences in cerebellar morphology, activation, and connectivity have been reported in many neuropsychiatric and neurodevelopmental disorders (see e.g. (53) for a review), including in ASDs (54, 55). Relevant for the current findings, double-blind, placebo-controlled studies in ADHD (56)

and depression (57) have found changes specifically in cerebellar activation and connectivity in groups of medication-naïve patients after just one dose of methylphenidate and SSRIs, respectively. Decreases in cerebellar blood perfusion after administration of antipsychotics in patients with schizophrenia (58), and changes in cerebellar volume in children with ADHD taking stimulants compared to medication-naïve ADHD patients (59) have also been reported. Similarly, prominent effects of psychotropic medication on the basal ganglia have been shown in studies employing fMRI (60) and positron emission tomography (56, 60) in schizophrenia and ADHD. Although the present findings may be related to these earlier results, a comprehensive functional interpretation of the regional specificity of underconnectivity between cerebellum and basal ganglia in our ASD-on group remains unclear.

Limitations and recommendations for future studies

The current study was not designed a priori to investigate effects of psychotropic medications. It was therefore only possible to look at differences between groups of ASD participants coarsely defined by whether or not they reported psychotropic medication usage at the time of their enrollment in the study, including during the fMRI scan. The medications taken by the participants, grouped together for the analysis here, fall into pharmacological categories with vastly different intended effects on brain function, emotions, and behavior. For instance, while stimulants act to increase dopamine levels in the brain, antipsychotics act to block dopamine receptors, theoretically exerting an opposing effect. High rates of polypharmacy in ASD further complicate efforts to control for the effects of psychotropic medications (7). Additionally, the current study took into account use of psychotropic medication at the time of the fMRI scan only, aggregating across medication type. No information on dosage or duration of treatment was available. It is likely that history of psychotropic medication use, and differences in dosage and duration of treatment also affect functional connectivity.

Ideally, the effect of different psychotropic medications would be studied in isolation, in fully controlled conditions (medication-naïve, on medication, off medication). This, of course, is ethically and pragmatically difficult to achieve, outside of formalized clinical trials, and was not feasible in the current study. It is furthermore challenging to fully model medication effects statistically, due to the numerous different types of psychotropic medications prescribed for children with ASDs. Such limitations will be unavoidable in most imaging studies, as caregivers will usually not be asked to take steps (such as withdrawing their child from medication) that may reduce the rate of volunteer participation and could affect the health and well-being of a child. Given demands for the study of large clinical samples, it is further problematic to exclude participants who are taking psychotropic medications from ASD research. Since more than half of all children with ASDs in the US today may be prescribed psychotropic medication, such medication-free samples would not be representative of the larger ASD population. Instead, we suggest that more detailed information regarding the medical history of past and current psychotropic medication usage (including dosages, duration, and side effects experienced) be collected, reported and, if possible, included in statistical analyses in the future.

Conclusions

Our findings suggest that medication use in children and adolescents with ASD may be associated with changes in functional connectivity. However, studies with explicit designs to fully control medication status and history will be needed to unravel differential neural effects of psychotropic medications on functional connectivity in ASDs. We hope that the results reported here will raise awareness that medication status may have a substantial effect on functional imaging findings in ASDs, and therefore needs to be well documented and, if possible, included in statistical tests.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Behavioral and cognitive differences in the ASD group taking psychotropic medication (ASD-on; n=25) compared to ASD participants who are medication free (ASD-none; n=24), shown separately for subscales of the ADOS- 2 and ADI-R measuring social-communicative behavior (A), overall autism symptom severity (C), and repetitive behaviors (D). Results for the BRIEF are shown in panel B. Error bars are standard error; * indicates significant differences at $p < .05$.

Figure 2.

Pairwise ROI connectivity differences, A) thresholded at $p < .05$, uncorrected, and B) thresholded at $p < .05$, FDR-corrected for multiple comparisons, except for ASD-on > ASD-none results which are shown at $p < .001$, uncorrected. Blue colors indicate under- and red colors indicate overconnectivity (t -values) in the respective group contrast. All ROIs are described in Table S1 in the Supplementary Information.

Table 1

Participant demographics

Gender	ASD-on (<i>n</i> = 25)			ASD-none (<i>n</i> = 24)			TD (<i>n</i> =50)		
	3 female			5 female			10 female		
	6 left			2 left			8 left		
Handedness	Mean (SD)	Range		Mean (SD)	Range		Mean (SD)	Range	<i>F</i> , <i>p</i> -value
Age in years	13.9 (3.0)	7.4–18.0		13.4 (2.5)	9.2–17.7		13.3 (2.8)	8.0–17.6	0.43, <i>p</i> =0.65
RMSD	0.06 (0.03)	0.02–0.11		0.06 (0.03)	0.02–0.11		0.06 (0.03)	0.02–0.14	0.32, <i>p</i> =0.73
WASI-II									
Verbal IQ	105 (18.6)	59–147		101 (16.7)	70–127		108 (9.7)	87–133	2.34, <i>p</i> =0.10
Nonverbal IQ	108 (18.7)	67–140		106 (20.4)	53–145		106 (13.4)	62–137	0.10, <i>p</i> =0.90
Full-scale IQ	107 (17.6)	61–141		104 (17.5)	66–130		108 (11.1)	79–132	0.57, <i>p</i> =0.57
ADOS-2									<i>t</i> , <i>p</i> -value
Social Affect	10.1 (4.2)	3–17		9.9 (3.6)	5–20		–	–	–0.20, <i>p</i> =0.85
Restricted/Repetitive Behaviors	3.5 (1.7)	1–7		2.5 (1.6)	0–5		–	–	–1.99, <i>p</i> =0.05
Total	14.1 (4.2)	7–21		12.4 (4.6)	5–24		–	–	–1.25, <i>p</i> =0.22
Severity	7.6 (1.8)	4–10		6.9 (2.2)	3–10		–	–	–1.12, <i>p</i> =0.27
ADI-R									
Social Interaction	17.0 (5.4)	1–24		18.9 (5.2)	6–28		–	–	1.30, <i>p</i> =0.20
Communication	12.6 (5.4)	2–22		14.1 (4.8)	4–24		–	–	0.96, <i>p</i> =0.34
Repetitive Behavior	6.1 (2.4)	3–12		5.7 (2.2)	1–9		–	–	–0.69, <i>p</i> =0.49

Table 2

Psychotropic medication use in ASD-on group

Participant	Stimulants	Mood Stabilizers/	SSRI/Antidepressants	Anxiolytics/Other ²	List of medications
1	+				Adderall
2		+		+	Risperdal, Ability, Xanax, Hydroxyzine
3	+		+		Concerta, Zolofl
4			+		Celexa
5	+	+	+		Risperdal, Zolofl, Concerta
6		+	+		Risperdal, Prozac
7	+			+	Adderall, Guanfacine
8	+	+	+		Risperdal, Methylphenidate, Celexa
9	+		+		Adderall, Sertraline
10		+	+	+	Ability, Trileptal, Clonidine, Prozac
11	+	+	+	+	Adderall, Lamictal, Xanax, Zolofl
12		+		+	Geodone, Guanfacine
13	+			+	Vyvanse, Guanfacine
14	+		+		Vyvanse, Effexor
15	+				Adderall
16	+	+	+	+	Depakote, Concerta, Prozac, Lamictal
17	+		+	+	Vyvanse, Guanfacine, Zolofl
18			+		Lexapro
19				+	Clonidine

Participant	Stimulants	Mood Stabilizers ¹	SSRI/Antidepressants	Anxiolytics/Other ²	List of medications
20		+	+		Abilify, Celexa
21		+			Abilify
22	+		+	+	Guanfacine, Concerta, Prozac
23		+			Seroquel, Lithium
24			+		Prozac
25	+	+	+	+	Risperdal, Guanfacine, Vyvanse, Trazodone
Total	14	12	16	11	

¹ Mood Stabilizers include antipsychotics and anticonvulsants

² Other include sympatholytics and an antihistamine (used as an anxiolytic)

Table 3

Comorbid conditions in participants with ASDs, n (%)

	ADHD	Depression	Anxiety	At least one comorbidity
ASD-on	10 (40%)	5 (20%)	8 (32%)	15 (60%)
ASD-none	1 (4%)	–	2 (8%)	3 (13%)

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