Atypical 'cross-talk' between mentalizing and mirror neuron networks in autism spectrum disorder

Inna Fishman, PhD1, Christopher L. Keown, MA1, Alan J. Lincoln, PhD2, Jaime A. Pineda, PhD3, and Ralph-Axel Müller, PhD1

1San Diego State University, San Diego, CA
2Alliant International University, San Diego, CA
3University of California San Diego, La Jolla, CA

Corresponding Author:
Inna Fishman, Ph.D.
Brain Development Imaging Laboratory
Department of Psychology
San Diego State University
6363 Alvarado Ct., Suite 200
San Diego, CA 92120
Phone: +1-619-594-2299
Email: ifishman@mail.sdsu.edu

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Importance: Converging evidence indicates that brain abnormalities in autism spectrum disorders (ASD) involve atypical network connectivity, but it is unclear whether altered connectivity is especially prominent in brain networks that participate in social cognition.

Objective: To investigate whether adolescents with ASD show altered functional connectivity (FC) in two brain networks involved in social processing and putatively impaired in ASD, the mentalizing (theory of mind; ToM) and mirror neuron systems (MNS).

Design: Cross-sectional study using resting-state functional magnetic resonance imaging (rs-fMRI).

Participants: Twenty-five adolescents with ASD between the age of 11 and 18 years, and 25 typically developing (TD) adolescents, matched for age, handedness and non-verbal IQ.

Main Outcome Measures: Statistical parametric maps testing the degree of whole-brain functional connectivity and social functioning measures.

Results: Relative to TD controls, participants with ASD showed a mixed pattern of both over- and underconnectivity in the ToM network, which was associated with greater social impairment. Increased connectivity in the ASD group was detected primarily between the regions of the MNS and ToM, and was correlated with sociocommunicative measures, suggesting that excessive ToM-MNS cross-talk might be associated with social impairment. In a secondary analysis comparing a subset of the 15 ASD participants with most severe symptomatology and a tightly matched subset of 15 TD controls, participants with ASD showed exclusive overconnectivity effects, in both ToM and MNS networks, which were also associated with greater social dysfunction.

Conclusions and Relevance: Adolescents with ASD showed atypically increased functional connectivity involving the mentalizing and mirror neuron systems, largely reflecting greater cross-talk between the two. This finding is consistent with recently emerging evidence of reduced network segregation in ASD and challenges the prevailing theory of general long-distance underconnectivity in ASD. This excess ToM-MNS connectivity may reflect immature or aberrant developmental processes in two brain networks involved in understanding of others, a domain of impairment in ASD. Further, robust links with sociocommunicative symptoms of ASD implicate atypically increased ToM-MNS connectivity in social deficits observed in ASD.
Humans are an inherently social species: our survival and success depend on our ability to navigate and thrive in complex social situations. This core ability is commonly impaired in autism spectrum disorder (ASD), a neurodevelopmental disorder affecting as many as 1 in 88 children.\(^1\) Despite the highly heterogeneous symptom manifestation, impairments in social functioning – including diminished social responsiveness, difficulty relating to others and recognizing others’ emotions and intentions – are a defining feature of ASD.\(^2\) These social deficits are considered the most universal and specific characteristics of ASD,\(^3\) both defining and distinguishing it from other developmental disorders.\(^4\) Yet, the neural mechanisms underlying social impairments remain largely undefined, despite attracting a great deal of research.

Currently, two – debatably related – prominent theories account for social dysfunction in ASD: theory of mind (ToM) and the mirror neuron system (MNS). ToM, also known as the mentalizing system, refers to the ability to infer contents of other people’s minds, including their beliefs and intentions. This ability to attribute mental states, or to mentalize, is impaired, or at the least delayed in ASD,\(^5\) giving rise to the “mindblindness” theory of autism.\(^6\) The MNS refers to the brain ‘mirror’ mechanisms that allow us to understand meaning of the actions and emotions of others by internally simulating and replicating them (as inferred from the original discovery in macaques of neurons firing during both action execution and observation\(^10\)). Evidence showing that imitation, a behavioral correlate of the MNS,\(^11\) is impaired in ASD (reviewed in ref.\(^12\)) has given rise to the dominant theory that atypical MNS functioning may be a key to understanding the nature of social deficits in ASD\(^13\)-\(^15\) (although see ref.\(^16\) for alternative views).

Even though both ToM and the MNS are involved in understanding others, a meta-analysis of more than 200 fMRI task-based activation studies\(^17\) confirmed that, functionally and anatomically, those are two distinct systems: while the MNS is an action understanding system, activated only in the presence of biological motion (i.e., when moving body parts such as hands or face are observed), ToM is recruited during a more abstract processing of others’ intentionality, in the absence of any biological motion. Although it is understood that judging others in real world likely involves both ToM and MNS, the functional distinction between them determined by this meta-analysis was adapted here. Anatomically, the meta-analysis identified ToM with a frontal-posterior network of brain regions, including the medial prefrontal cortex (mPFC), the bilateral temporal-parietal junction (TPJ), and the posterior cingulate cortex/precuneus (PCC), while the human MNS engaged the anterior intraparietal sulcus (aIPS; also referred to as the rostral inferior parietal lobule, IPL), premotor cortex (PMC; also referred to as the caudal inferior frontal gyrus, IFG), and posterior superior temporal sulcus (pSTS).\(^17\)

While neuroimaging and electrophysiological evidence suggests that ASD is associated with localized abnormalities in certain ToM\(^18\)-\(^19\) and MNS\(^20\)-\(^23\) brain areas, it is also becoming increasingly evident that ASD is characterized by abnormal connectivity throughout the brain,\(^24\)-\(^29\) presumed to stem from altered neurodevelopmental trajectories.\(^28\)-\(^29\) Widespread abnormalities in interregional connections in ASD have been predominantly demonstrated with functional connectivity magnetic resonance imaging (fcMRI) assessing functional coordination between spatially distributed brain regions.\(^27\) Functional connectivity (FC) – inferred from interregional cross-correlations of the blood oxygen level-dependent (BOLD) signal – can be detected even at rest, in the absence of an overt cognitive task (as reviewed in ref.\(^30\)). Importantly, those patterns correspond to brain networks recruited during specific cognitive or mental processes\(^31\)-\(^34\) and are, therefore, thought to reflect intrinsically organized functional networks\(^35\) formed by a long history of frequent coactivation associated with functional specialization.\(^36\)-\(^37\) Moreover, resting state FC patterns are largely consistent with anatomical connectivity\(^38\)-\(^39\) and appear robust and highly reliable across individuals.\(^39\)-\(^43\)

The present study investigated whether adolescents with ASD show altered FC in the MNS and ToM, two brain networks involved in social processing and putatively impaired in ASD, by using resting-state fcMRI to assess inter-regional BOLD correlations in these networks. Our aims were two-fold: to examine the extent of functional specialization, as deduced from the functional connectivity, of the ToM and MNS networks in adolescents with ASD (i.e., whether the two networks are functionally segregated), and to relate FC of these networks involved in understanding others to variation on clinical measures of social impairment. It was hypothesized that individuals with ASD would exhibit aberrant connectivity within and between these networks, compared to matched typically developing (TD) controls, and that those with greatest social impairments within the ASD group would show the most atypical connectivity patterns.

**Methods**

**Participants.** Thirty adolescents with ASD and 26 TD adolescents, between 11 and 18 years of age, were enrolled in the study. After excluding five ASD participants due to excessive head motion (>15% of time points) and one TD adolescent due to hardware malfunction, the final sample included 25 ASD and 25 TD participants matched for age, handedness and non-verbal IQ (Table 1; eMethods; eTable 1). ASD diagnoses were established using the Autism Diagnostic Interview-Revised (ADI-R),\(^44\) the Autism Diagnostic Observation Schedule (ADOS),\(^45\)


and expert clinical judgment (by co-author AJL) according to DSM-IV criteria. History of autism-related medical conditions (e.g., epilepsy, Fragile-X, tuberous sclerosis) served as an exclusionary criterion. Inclusion in the TD group required absence of personal or family history of autism, and of personal history of any other neurological or psychiatric conditions. All participants had verbal and nonverbal IQ scores > 70, as assessed by the Wechsler Abbreviated Scale of Intelligence (WASI). In addition to the ADI- and ADOS-derived indices of social behavior available only for ASD participants, social functioning was also assessed in all participants using the Social Responsiveness Scale (SRS), an informant-based rating scale measuring social impairments characteristic of ASD; it was administered to the participants’ parents. Hand preference was assessed with the Edinburgh Handedness Inventory. Informed assent and consent was obtained from all participants and their caregivers in accordance with the institutional review boards of the University of California, San Diego and San Diego State University.

**MRI data acquisition.** Imaging data were acquired on a GE 3T MR750 scanner with an 8-channel head coil. High-resolution anatomical images were obtained using a standard T1-weighted fast SPGR sequence (TR = 11.08 ms; TE = 4.3 ms; flip angle = 45°; field of view [FOV] = 256 mm; 256 x 256 matrix; 180 slices; 1 mm³ resolution). Functional T2*-weighted echo-planar images were acquired in one, 6:10 minute resting-state scan consisting of 185 whole-brain volumes (TR = 2000 ms; TE = 30 ms; flip angle = 90°; FOV = 220 mm; 64 x 64 matrix; 3.4 mm² in-plane resolution; 3.4 mm slice thickness; 42 axial slices covering the whole brain). Throughout the scan, participants were instructed to keep their eyes on a white fixation cross displayed in the center of a screen.

**fMRI data preprocessing.** Images were processed primarily using Analysis of Functional NeuroImages (AFNI). The first five frames were discarded to remove signal equilibration effects, resulting in 180 total whole brain volumes. Functional data were slice-time and motion corrected by realigning to the first time point, field-map corrected to remove distortions resulting from magnetic field inhomogeneity, co-registered to the anatomical image using a single transformation matrix, resampled to 3.0 mm isotropic voxels, standardized to the N27 Talairach template and spatially smoothed with an isotropic Gaussian filter to an effective full-width at half-maximum (FWHM) of 6 mm. The resulting images were then band-pass filtered at .008 < f < .08 Hz to isolate frequencies at which intrinsic network-specific BOLD correlations predominated.

To minimize the confounding effects of head motion on BOLD correlations, six scan-to-scan rigid-body motion parameters (three rotations, three translations) estimated from realignment of functional volumes were modeled as nuisance variables and removed with regression, along with the mean white matter (WM) and ventricular (CSF) signals extracted from the masks derived from Freesurfer’s automated segmentation of anatomical images into tissue compartments, and reduced by one voxel in all directions (all regressors band-pass filtered at .008 < f < .08 Hz). Time points with excessive head motion (head displacement >1.5 mm, computed as the root sum of square of displacement between any two time points) and their immediately preceding and following time points were censored from further analyses; blocks of time points with <10 usable consecutive images were also excluded. Based on this criterion, the mean percentage of data censored from all 50 participants was <1%. Percentage of data censored did not differ between groups ($M_{\text{ASD}} = 0.71\%$; $M_{\text{TD}} = 0.67\%$; $t(1,48) = 0.06, p = 0.95$). Finally, the root mean square of displacement (RMSD) across the entire time series calculated for each participant did not differ between the groups ($M_{\text{ASD}} = 0.133$; $M_{\text{TD}} = 0.125$; $t(1,48) = 0.23, p = 0.82$), and was not significantly correlated with age ($p = 0.13$) or full scale IQ ($p = .16$).

**ToM and MNS regions of interest.** Seeds were placed in regions found to be consistently activated by mentalizing or mirror neuron tasks, as determined by meta-analysis, including four ToM seeds: the mPFC, the right and left TPJ, and the PCC, and six MNS seeds: the bilateral aIPS, pSTS, and PMC (see Figure 1, left panel for seed placements and Talairach coordinates). Seeds were created using the Talairach-Tournoux Stereotaxic Atlas in AFNI as 6 mm radius spheres, covering 33 voxels in 3 mm³ space.

**fcMRI analyses.** Following fMRI preprocessing and removal of nuisance variables, the average BOLD time course was extracted from each seed and correlated with the time courses of all voxels across the brain (whole-brain voxel-wise correlations), for every participant. The resulting correlation coefficients were converted to normally distributed z-values (using Fisher’s r-to-z transformation) and entered into one- and two-independent-sample(s) t-tests to examine within- and between-group FC effects. All statistical maps were corrected for multiple comparisons to a cluster-size-corrected threshold of $p < 0.05$, using Monte Carlo simulation.

**Summary connectivity scores and correlations with clinical measures.** To examine relationships between social impairment and functional connectivity, within- and between-network connectivity indices were computed by averaging $z$ scores for all within- and between-network ROI pairs, respectively. To minimize multiple comparisons (and associated Type 1 error), four $a$ priori selected social functioning measures were chosen for the correlational analyses with connectivity indices within the ASD cohort: three diagnostic scores (two ADI-R socio-communicative components: ADI-Social and ADI-Communication, and the ADOS Communication + Social [CS] total score) and one parental report sociability score (SRS Total). Relationship between FC and these four measures were examined using Spearman correlations due to the skewed distributions of the clinical measures. Because neither connectivity
indices, nor social measures were significantly correlated with age \([all \, rs < .22, \, all \, ps > .56]\), age was excluded from any further analyses.

**Results**

*Whole-brain connectivity.* Results from the whole-brain within-group FC analyses performed for each of the 10 seeds are summarized in Figure 1 (see supplementary eTable 2 and eTable 3 for detailed descriptions, including peak coordinates). Direct group comparisons (corrected \(p < .05\)) revealed no significant between-group differences in FC for any of the MNS seeds, but several significant clusters of differential connectivity for the ToM network, including underconnectivity (TD > ASD) of the bilateral TPJ with the bilateral superior temporal gyri (STG) and PCC/PC, and overconnectivity (ASD > TD) of the mPFC and PCC with superior parietal lobule (SPL) and middle and inferior frontal gyri (MTG and IFG; Figure 2A and Table 2).

*Summary connectivity indices and their relationship to clinical measures.* Given this mixed pattern of both weaker and stronger BOLD correlations in the ToM, its connectivity was summarized with two separate indices calculated by averaging \(z\) scores for significantly under- and overconnected clusters, respectively. Because no significant clusters emerged in direct between-group comparison of the MNS, its mean connectivity was computed by averaging \(z\) scores for all MNS ROI pairs. Finally, mean ToM-MNS between-network connectivity was estimated by averaging \(z\) scores for all between-network ROI pairs. A correlational matrix of four connectivity indices \(x\) 4 social measures yielded a Bonferroni adjusted \(p < 0.05/16 = 0.003\). Significant correlations were detected between ASD social symptoms and the extent of ToM overconnectivity (Table 3): namely, mean \(z\) score for ToM overconnected clusters was correlated with ADI-Social and ADI-Communication scores \((r = .45, p < .05\) and \(r = .51, p < .01\), respectively), although neither survived Bonferroni correction for multiple comparisons. While no significant FC group differences were detected for the MNS network, its average connectivity was positively correlated with ADI-Social scores \((r = .50, p = .013\), uncorrected\) such that greater MNS connectivity was associated with increased social symptoms of ASD. Further, the ToM-MNS between-network connectivity was significantly correlated with ADI-Social scores \((r = .58, p = .003)\), indicating that greater ToM-MNS cross-talk (i.e., atypically increased connectivity and reduced segregation between networks) was associated with more severe social impairment.

*Post-hoc analysis: Replication and robustness of findings in ASD subset with most severe symptomatology.* Based on these positive relationships between symptom severity and MNS and ToM-MNS functional connectivity, a post hoc FC analysis was performed in a subset of ASD participants \((n = 15)\) with highest level of social symptomatology as defined by ADOS CS scores \(\geq 10\) (see eFigure 1 for within-group connectivity maps). Direct group comparison of this ASD subsample and 15 TD participants optimally matched on age, motion, and IQ (see eTable 4) corroborated earlier results of increased connectivity (ASD > TD) of the mPFC and PCC regions of ToM, but also revealed increased – rather than weaker – connectivity (ASD > TD) of the right TPJ region of ToM (Figure 2B; Table 4). Notably, this analysis yielded a significant between-group difference in the MNS network – which was absent in the direct comparison of total samples – with greater connectivity (ASD > TD) between right aIPS and left superior frontal gyrus and PCC (Figure 2B; Table 4). Finally, consistent with analyses for the entire cohort, positive correlation was detected between greater ToM-MNS between-network connectivity and ADI-Social scores and \((r = .56, p = .04;\) Figure 2B, right panel), although it did not survive Bonferroni correction for multiple comparisons.

**Discussion**

We used rs-fcMRI to investigate functional connectivity in two brain networks crucial for social processing (ToM and MNS) in adolescents with ASD, relative to TD controls. In contrast to previous findings of predominantly reduced connectivity in ASD detected at rest in other functional networks,\(^{56-58}\) a mixed pattern of both over- and underconnectivity was observed in the ToM network. Namely, relative to TD participants, adolescents with ASD showed enhanced connectivity between mPFC and the superior parietal lobule (SPL), precuneus (PC), and right posterior middle temporal gyrus, as well as between PC/PCC and the right middle and inferior frontal gyri. On the other hand, the ASD group showed weaker connectivity between the bilateral TPJ and PCC and superior temporal gyrus, including pSTS.

An unexpected finding was the lack of significant between-group differences in the MNS functional connectivity. However, when directly comparing a subset of the ASD participants with most severe socio-communicative symptoms and a matched TD subsample, overconnectivity was detected between the rIPS region of the MNS and PCC, as well as between rIPS and the left superior frontal gyrus. This secondary analysis involving only the ASD participants with greatest symptom severity also revealed overconnectivity in three ToM seeds, namely between the rTPJ and the left middle frontal gyrus, mPFC and the bilateral superior and middle frontal gyri, and PCC and the right middle frontal gyrus and left IFG. Remarkably, no underconnectivity effects were observed
for this more homogeneous ASD subsample; instead, increased connectivity was detected for both MNS and ToM networks. These findings appear inconsistent with the theory of generally reduced long-distance connectivity in ASD, or the more specific hypothesis of fronto-parietal underconnectivity.

Critically, close examination of the regional specificity of these findings – observed both in the entire sample and in the subset of participants with greatest symptom severity – revealed that atypical connectivity in ASD occurred between the regions of the MNS and ToM: for instance, in the analysis of the entire sample, the bilateral TPJ region of ToM showed reduced connectivity with the superior temporal gyrus, which included the pSTS region of the MNS. Similarly, clusters found to be overconnected with precuneus – a ToM seed – contained IFG, a canonical MNS region. Likewise, in the secondary subset analysis, clusters that emerged as significantly overconnected in both MNS and ToM networks also contained regions from the other network (Table 4); for instance, the rIPS seed of the MNS was overconnected (ASD > TD) with the PCC region of ToM. This pattern of atypical ToM-MNS cross-talk suggests that the two social brain systems putatively impaired in ASD are less functionally segregated from one another in adolescents with ASD. This is in contrast with typical development, during which functional brain networks become simultaneously more integrated (within-network connections strengthen) and segregated (between-network connections weaken). Thus, the excess ToM-MNS connectivity observed in ASD may reflect immature or aberrant developmental processes in two brain networks involved in understanding of others. Notably, this finding of atypical ToM-MNS cross-talk is consistent with recently emerging evidence of reduced network segregation in ASD.

Overconnectivity was most pronounced in a subsample of 15 ASD participants with highest symptom severity. As one possibility, cross-talk between ToM and MNS, which largely accounted for the overconnectivity effects, might reflect a compensatory mechanism involving strengthening of the atypical connections secondary to social deficits. Specifically, the dynamic nature and complexity of social stimuli and social interactions may be overtaxing for inefficient neural networks in ASD; as a result, overconnectivity may be a consequence of an overutilization of aberrant social circuits. The observed links between ToM-MNS cross-network connectivity and socio-communicative symptom severity may support this interpretation. At the very least, these findings suggest that connectivity of, and between, the ToM and MNS networks plays a role in autistic symptomatology.

The detection of ToM overconnectivity in ASD is particularly noteworthy given the findings indicating reduced activation in the key ToM regions in ASD (e.g., refs. 18,57,66-67). On the other hand, greater ToM connectivity in ASD might be in line with evidence of reduced specialization of mentalizing brain regions in autism as demonstrated by activation for tasks that do not pertain to ToM. The ToM network is considered crucial for maneuvering in social contexts, as it supports the understanding of other people’s intentions and beliefs. Thus, our finding of ToM overconnectivity in ASD, especially in participants with greater symptom severity, may indicate a state of heightened activity associated with reduced efficiency and behavioral impairment in this domain.

Our second hypothesis regarding links between atypical patterns of connectivity and social symptom severity was also supported: Robust positive correlations were detected between ToM and MNS overconnectivity and ASD socio-communicative symptoms, as measured by the ADI-R Social and Communication scales, indicating that those with greater social impairment had more increased connections within and between these networks. In particular, the relationship between increased socio-communicative symptoms and excessive – rather than reduced – ToM-MNS connectivity is consistent with the notion that social dysfunction is associated with inadequate segregation between the two social networks.

While suggesting links between ToM and MNS connectivity and social impairment in ASD, our findings cannot establish causality. Atypical functional connectivity of these networks could reflect neurobiological abnormalities contributing to the emergence of social impairment. However, alternatively, abnormal social development in children with ASD may result in aberrant connectivity. This latter possibility is supported by evidence that network connectivity is affected by learning and experience-driven plasticity. Our findings may also reflect a combination of early causative and secondary, experience-driven effects. Notable in this context was the absence of correlations between connectivity measures and ADOS and SRS scores, both of which represent current abilities, contrasted by sizeable correlations between connectivity and ADI-R scores representing the early history of socio-communicative impairment. While caution is required, given the non-experimental nature of these measures, this pattern of findings could suggest that at least some of the atypical ToM and MNS connectivity observed here may reflect neural abnormalities possibly contributing to the early emergence of the disorder.

Among limitations of the present study is exclusion of low-functioning adolescents with ASD, due to the extreme sensitivity of fMRI to head motion. While head motion is clearly also an issue in studying high-functioning children, we used a number of procedures beyond conventional motion correction to minimize the effects of head movement. With this in mind, it cannot be determined whether our findings also apply to the lower end of the autistic spectrum.

In sum, the current results demonstrate atypical connectivity of and between ToM and MNS networks in
adolescents with ASD, predominantly reflected in overconnectivity. Moreover, the extent of atypical connectivity was correlated with greater social dysfunction, suggesting that abnormal neural connections involving the mentalizing and mirror neuron systems are related to the social impairments observed in ASD.

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Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ASD (n = 25)</th>
<th>TD (n = 25)</th>
</tr>
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<tbody>
<tr>
<td>Gender (M/F)</td>
<td>22/3</td>
<td>20/5</td>
</tr>
<tr>
<td>Handedness (R/L)</td>
<td>23/3</td>
<td>21/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>14.8 (1.8)</td>
<td>14.4 (1.5)</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>111 (15)</td>
<td>106 (10)</td>
</tr>
<tr>
<td>Non-verbal IQ</td>
<td>111 (16)</td>
<td>108 (11)</td>
</tr>
<tr>
<td>Full-scale IQ</td>
<td>113 (15)</td>
<td>108 (10)</td>
</tr>
<tr>
<td>ADOS Communication</td>
<td>2.9 (1.4)</td>
<td>n/a</td>
</tr>
<tr>
<td>Social Interaction</td>
<td>7.6 (3.2)</td>
<td>n/a</td>
</tr>
<tr>
<td>Repetitive Behavior</td>
<td>2.0 (1.4)</td>
<td>n/a</td>
</tr>
<tr>
<td>ADI-R Social Interaction</td>
<td>16.5 (6.2)</td>
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</tr>
<tr>
<td>Communication</td>
<td>12.6 (6.2)</td>
<td>n/a</td>
</tr>
<tr>
<td>Repetitive Behavior</td>
<td>6.0 (2.3)</td>
<td>n/a</td>
</tr>
<tr>
<td>SRS, Total</td>
<td>78.5 (9.8)</td>
<td>41.5 (5.1)</td>
</tr>
</tbody>
</table>

Note: IQ, intelligence quotient; ADOS, Autism Diagnostic Observation Schedule; ADI-R, Autism Diagnostic Interview-Revised; SRS, Social Responsiveness Scale. Four out of 25 ASD participants met either the ADOS or the ADI-R cutoff, while meeting the clinical diagnostic criteria as judged by expert clinical judgment (21/25 participants met both ADOS and ADI-R cutoffs). Twelve ASD participants presented with comorbid psychiatric conditions, including ADHD (5), OCD (2), depression (3) and anxiety (4), with 2/12 diagnosed with more than one comorbid condition. Ten ASD participants were reported to be on psychoactive medications, as detailed in the Supplemental Material: eMethods and eTable 1.

Table 2. Regions Exhibiting Group Differences (ASD vs. TD) in Functional Connectivity, separately for MNS and ToM seeds

<table>
<thead>
<tr>
<th>Seed</th>
<th>Peak Location</th>
<th>Talairach coordinates</th>
<th>Cluster Volume (μl)</th>
<th>T-score</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>x  y  z</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laIPS</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>raIPS</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPMC</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rPMC</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lpSTS</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rpSTS</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITPJ</td>
<td>L Superior Temporal Gyrus / pSTS</td>
<td>-50 -28 12</td>
<td>918</td>
<td>4.64</td>
</tr>
<tr>
<td>rTPJ</td>
<td>R/l Superior Temporal Gyrus / pSTS</td>
<td>-56 -26 12</td>
<td>2808</td>
<td>4.60</td>
</tr>
<tr>
<td>mPFC</td>
<td>L CCD / SPL</td>
<td>-14 -62 50</td>
<td>1755</td>
<td>-4.82</td>
</tr>
<tr>
<td>PCC</td>
<td>R Middle Frontal Gyrus, IFG</td>
<td>38 26 38</td>
<td>999</td>
<td>-5.17</td>
</tr>
</tbody>
</table>

Note: laIPS = left anterior intraparietal sulcus (Talairach coordinates -40 -40 45); raIPS = right anterior intraparietal sulcus (40 -40 45); IPMC = left premotor cortex (-40 5 40); rPMC = right premotor cortex (40 5 40); lpSTS = left posterior superior temporal sulcus (-50 -55 10); rpSTS = right posterior superior temporal sulcus (50 -55 10); ITPJ = left temporal-parietal junction (-50 -55 25); rTPJ = right temporal-parietal junction (50 -55 25); mPFC = medial prefrontal cortex (0 50 20); PCC = posterior cingulate cortex/precuneus (0 -60 40); SPL = Superior Parietal Lobule; IFG = Inferior Frontal Gyrus; MNS: mirror neuron system; ToM: theory of mind; L: left; R: right.
Table 3. Correlations between connectivity indices and social symptoms measures in participants with ASD

<table>
<thead>
<tr>
<th></th>
<th>ADOS-CS</th>
<th>ADI-R, Social</th>
<th>ADI-R, Comm.</th>
<th>SRS Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ToM Overconnectivity (PCC, mPFC)</td>
<td>-.29</td>
<td>.45*</td>
<td>.51**</td>
<td>-.04</td>
</tr>
<tr>
<td>ToM Underconnectivity (bilateral TPJ)</td>
<td>-.14</td>
<td>.22</td>
<td>.05</td>
<td>-.42</td>
</tr>
<tr>
<td>MNS Connectivity</td>
<td>-.10</td>
<td>.50*</td>
<td>.47*</td>
<td>-.26</td>
</tr>
<tr>
<td>ToM-MNS Between-Network Connectivity</td>
<td>-.07</td>
<td>.58***</td>
<td>.57**</td>
<td>.05</td>
</tr>
</tbody>
</table>

Note: ***p < .003 (Bonferroni corrected p < .05); **p < .01 (uncorrected); *p < .05 (uncorrected); all correlation coefficients are calculated with Spearman rank correlations for n = 25.

Table 4. Regions Exhibiting Group Differences in Functional Connectivity, in a subsample of 15 ASD participants with ADOS-CS ≥ 10 and 15 TD controls, for MNS and ToM seeds.

<table>
<thead>
<tr>
<th>Seed</th>
<th>Peak Location</th>
<th>Talairach coordinates (μl)</th>
<th>Cluster Volume (μl)</th>
<th>T-score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x  y z</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laIPS</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>raIPS</td>
<td>L Superior Frontal Gyrus</td>
<td>-16 26 48</td>
<td>1296</td>
<td>-4.41</td>
</tr>
<tr>
<td></td>
<td>R/L PCC</td>
<td>2 -38 36</td>
<td>837</td>
<td>-3.86</td>
</tr>
<tr>
<td>rPMC</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lpSTS</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rpSTS</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iTPJ</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rTPJ</td>
<td>L Middle Frontal Gyrus</td>
<td>-16 46 30</td>
<td>756</td>
<td>-4.30</td>
</tr>
<tr>
<td>mPFC</td>
<td>L Middle / Superior Frontal Gyrus</td>
<td>-26 -10 44</td>
<td>2808</td>
<td>-4.12</td>
</tr>
<tr>
<td></td>
<td>R Superior / Middle Frontal Gyrus</td>
<td>28 -8 56</td>
<td>1944</td>
<td>-4.70</td>
</tr>
<tr>
<td></td>
<td>R Middle Frontal Gyrus</td>
<td>34 22 38</td>
<td>1269</td>
<td>-4.66</td>
</tr>
<tr>
<td></td>
<td>L IFG, p.Tri/p.Orb</td>
<td>-46 40 2</td>
<td>810</td>
<td>-4.51</td>
</tr>
</tbody>
</table>

Note: laIPS = left anterior intraparietal sulcus (Talairach coordinates –40 –40 45); raIPS = right anterior intraparietal sulcus (40 –40 45); lPMC = left premotor cortex (–40 5 40); rPMC = right premotor cortex (40 5 40); lpSTS = left posterior superior temporal sulcus (–50 –55 10); rpSTS = right posterior superior temporal sulcus (50 –55 10); iTPJ = left temporal-parietal junction (–50 –55 25); rTPJ = right temporal-parietal junction (50 –55 25); mPFC = medial prefrontal cortex (0 50 20); PCC = posterior cingulate cortex/precuneus (0 –60 40); IFG = Inferior Frontal Gyrus; MNS: mirror neuron system; ToM: theory of mind; L: left; R: right.
Figure 1. Within-group functional connectivity maps for MNS (top panel) and ToM (bottom panel) seeds. Results of the within-group (ASD, TD; \( p < .05 \) corrected) analyses obtained for each MNS and ToM seeds (top and bottom panels, respectively) are presented in a conjunction view. Seed ROIs are presented on the axial slices on the left (red dots reflect the actual size of the spherical ROIs). Inflated maps were generated using Surface mapping with AFNI (SUMA; http://afni.nimh.nih.gov/afni/suma).

laIPS = left anterior intraparietal sulcus (Talairach coordinates \(-40 -40 45\)); raIPS = right anterior intraparietal sulcus \((40 -40 45)\); lPMC = left premotor cortex \((-40 5 40)\); rPMC = right premotor cortex \((40 5 40)\); lpSTS = left posterior superior temporal sulcus \((-50 -55 10)\); rpSTS = right posterior superior temporal sulcus \((50 -55 10)\); ITPJ = left temporal-parietal junction \((-50 -55 25)\); rTPJ = right temporal-parietal junction \((50 -55 25)\); mPFC = medial prefrontal cortex \((0 50 20)\); PCC = posterior cingulate cortex/precuneus \((0 -60 40)\); L: left; R: right.

Figure 2. Regions exhibiting group differences (ASD vs. TD) in functional connectivity and relationship between functional connectivity and clinical severity in the ASD group.

(A) Clusters of significantly different functional connectivity \((p < .05 \) corrected) in ASD participants relatively to the TD participants are illustrated for the ToM seeds. Scatterplot on the right shows the relationship between the ToM-MNS overconnectivity (average \( z \) scores for all between-network ROI pairs) and social symptomatology measured by the ADI-Social scores (Spearman \( r(25) = .58, p = .003 \)).

(B) Clusters of significantly different FC \((p < .05 \) corrected) in the subset of 15 ASD participants with ADOS-CS \( \geq 10 \) and 15 matched TD participants. All depicted ToM and MNS seeds yielded overconnected clusters (ASD>TD). Scatterplot on the right shows the relationship between the ToM-MNS overconnectivity (average \( z \) scores for all between-network ROI pairs) and social symptomatology measured by the ADI-Social scores (Spearman \( r(15) = .56, p = .04 \)). Increasing ADI-Social values indicate greater social impairment.