Hyperconnectivity of the Right Posterior Temporo-parietal Junction Predicts Social Difficulties in Boys with Autism Spectrum Disorder

Hsiang-Yun Chien, Hsiang-Yuan Lin, Meng-Chuan Lai, Susan Shur-Fen Gau, and Wen-Yih Isaac Tseng

The posterior right temporo-parietal junction (pRTPJ) is a key brain region representing other’s mental status. Despite reports of atypical activation at pRTPJ during mentalizing in individuals with autism spectrum disorder (ASD), the intrinsic functional connectivity (iFC) of the pRTPJ remains under-investigated. We examined whether boys with ASD show altered resting-state iFC of the pRTPJ, and whether atypical iFC of the pRTPJ is associated with social deficits in ASD in a sample of 40 boys with high-functioning ASD (aged 9–17 years, mean age, 12.38 ± 2.17; mean IQ, 105.60 ± 16.06) and 42 typically developing (TD) boys (aged 9–17 years, mean age, 11.64 ± 2.71; mean IQ, 111.29 ± 13.45). Both groups received resting-state fMRI assessment after imaging data quality control for in-scanner head motion and spatial coverage. Seed-based approach was used to investigate iFC of the pRTPJ. TD and ASD boys demonstrated a resting-state pRTPJ iFC pattern comparable to the known spatial involvement of the default-mode network. Boys with ASD showed pRTPJ hyperconnectivity relative to TD boys in the right ventral occipito-temporal cortex. This atypically increased iFC in the ASD group was positively correlated with social deficits assessed by the Chinese version of the Autism Diagnostic Interview-Revised and the Social Responsive Scale. Our findings provide empirical support for functional “dysconnectivity,” that is, atypical functional integration among brain regions, as an integral component of the atypical neurobiology of ASD. Autism Res 2015, 8: 427–441. © 2015 International Society for Autism Research, Wiley Periodicals, Inc.

Keywords: autism spectrum disorder; right posterior temporo-parietal junction; resting-state fMRI; functional connectivity; ventral occipito-temporal cortex

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by difficulties in social interactions and communication. Difficulties in social reciprocity in ASD encompass several domains of social cognition and perception [Lai, Lombardo, & Baron-Cohen, 2014], including mentalizing difficulties [Frith, 2001; Senju, 2012], impaired dyadic social perception [Boucher, 2012; Pelphrey, Shultz, Hudac, & Vander Wyk, 2011], reduced social motivation [Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012], and anomalous self-referential cognition [Lombardo & Baron-Cohen, 2011]. Task-functional magnetic resonance imaging (fMRI) studies of ASD have collectively revealed anomalous activation in the so-called “social brain” regions during social-processing experiments [Dichter, 2012; Philip, Dauvermann, Whalley, Bayham, Lawrie, & Stanfield, 2012]. Moreover, emerging evidence indicates that atypical development in neural connectivity and synchrony across brain regions is associated with ASD [Minshew & Williams, 2007; Schipul, Keller, & Just, 2011; Uddin, Supekar, & Menon, 2013; Vissers, Cohen, & Geurts, 2012].

Intrinsic functional connectivity (iFC), represented by the correlation of low frequency (e.g., < 0.1 Hz) spontaneous fluctuations in neural activity measured by resting-state fMRI (rs-fMRI) blood-oxygen-level-dependent (BOLD) signal, characterizes the functional organization of the brain at a systems level [Fox & Raichle, 2007]. Previous iFC studies have demonstrated a preponderance of hypoconnectivity in anterior–posterior connection [Cherkassky, Kana, Keller, & Just, 2006], interhemispheric synchronization [Anderson et al., 2011], social-affective processing circuits [Alaerts, Woolley, Steyaert, Di Martino, Swinnen, & Wenderoth, 2015].

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principally involved in perspective taking [Spengler, von 
Kubit & Jack, 2013; Mars et al., 2012]. The pRTPJ is 
connected with areas largely overlapping with the DMN 
[Alaerts et al., 2014; Gotts et al., 2012; Saxe & Kanwisher, 
2003]. Unlike the selective response in neurotypical 
controls, pRTPJ is reported nonselectively activated during 
mentaling about self and others in adults with ASD, 
and the reduced selectivity of pRTPJ for mentalizing is 
associated with social difficulties measured by the 
Autism Diagnostic Interview-Revised (ADI-R) [Lombardo, 
Chakrabarti, Bullmore, MRC Aims Consortium, & 
Baron-Cohen, 2011]. The pRTPJ has also been shown 
activated atypically in moral judgment [Koster-Hale, 
Saxe, Dungan, & Young, 2013] and inferring intention 
[Kana, Libero, Hu, Deshpande, & Colburn, 2012] in 
adults with ASD. Adolescents and adults with ASD display 
weaker synchronization between pRTPJ and supplemental motor area [Kana et al., 2012] and frontal region 
[Kana, Keller, Cherkassky, Minshew, & Just, 2009] during 
mentaling task. Notably, Dufour et al. [2013], using different mentaling tasks and a larger sample 
size, yielded inconsistent results regarding pRTPJ impairment 
in ASD.

Despite its important role in social cognition, pRTPJ 
has received insufficient attention in the resting-state 
fundamental connectivity literature in ASD. Previous studies 
have reported reduced iFC of RTPJ with posterior 
cingulate cortex (PCC) and pSTS [Fishman et al., 2014] 
in adolescents with ASD. With a larger sample size, the 
current study aimed to specifically investigate the 
resting-state iFC of the posterior part of RTPJ in children and adolescents with ASD. We hypothesized that 
boys with high-functioning ASD would have atypical 
pRTPJ iFC, which would be linked with the severity of 
reciprocal social impairment.

Methods

Participants and Procedure

The Research Ethics Committee at the National Taiwan 
University Hospital (NTUH) approved this study prior to 
implementation (ID: 20090306; ClinicalTrials.gov number, NCT00916851). The procedures and purpose 
of the study were explained face-to-face to the participants 
and their parents, who then provided written informed consent. All participants underwent the same 
clinical and MRI assessments.

We recruited 48 Taiwanese boys with high-functioning ASD (aged 9–17 years, full-scale intelligence 
quotient (IQ) above 70) consecutively from the Department of Psychiatry, NTUH, and 48 typically developing 
(TD) boys (aged 9–17 years) from schools in similar geographical districts to the ASD group. All boys with ASD 
were clinically diagnosed according to the DSM-IV-TR and ICD-10 criteria by child psychiatrists, and further
confirmed by the Chinese version of the ADI-R [Gau et al., 2010; Rutter, Le Couteur, & Lord, 2003]. The corresponding author reviewed all the medical records and the ADI-R interviews again and found all these participants met the DSM-5 diagnostic criteria for ASD [American Psychiatric Association, 2013]. The Chinese version of the ADI-R, translated into Mandarin by Gau et al. [2010], was approved by Western Psychological Services in 2007.

TD boys were recruited if they did not have any current or lifetime DSM-IV psychiatric disorder based on the Chinese version of the K-SADS-E interview [Gau, Chong, Chen, & Cheng, 2005; Orvaschel, Puig-Antich, Chambers, Tabrizi, & Johnson, 1982] with the participants and their parents. Exclusion criteria for all participants included past or current neurological or severe medical illness, lifetime diagnoses of learning disorder, substance use disorder, schizophrenia, bipolar disorder, and major depression, current severe anxiety disorders, current use of psychotropic medication except psychostimulants (n = 5), and a full-scale IQ less than 70. Boys with ASD who had other definite concurrent DSM-IV Axis I disorders (except for attention-deficit hyperactivity disorder) based on the K-SADS-E interview were also excluded. None of the participants took any medication for at least 1 week before and during all assessments.

Intellectual function was assessed by the Wechsler Intelligence Scale for Children – 3rd edition (WISC-III) [Wechsler, 1991]. Handedness was assessed by the Edinburgh Inventory [Oldfield, 1971]. Beside the Chinese ADI-R interview, all parents completed the Chinese version of the Social Responsiveness Scale (SRS) [Gau, Liu, Wu, Chiu, & Tsai, 2013] to assess current social deficits. The Chinese SRS, translated by Gau et al. [2013], has well-accepted psychometric properties for measuring autistic features in the Taiwanese population [Hsiao, Tseng, Huang, & Gau, 2013]. The confirmatory factor analysis revealed a four-factor structure, namely “social communication,” “autism mannerism,” “social awareness,” and “social emotion” [Gau et al., 2013].

MRI Assessments

fMRI data acquisition. Data were obtained on a 3T scanner (Siemens Magnetom Tim Trio) with a 32-channel phased-arrayed head coil. All participants were verbally instructed to remain still with their eyes closed to complete a 6-min rs-fMRI scan. The imaging parameters were 180 echo planar imaging (EPI) volumes; TR = 2,000 ms; TE = 24 ms; flip angle = 90°; field of view (FOV) = 256 × 256 mm2; matrix size = 64 × 64; 34 axial slices acquired in an interleaved descending order; slice thickness = 3 mm; voxel size = 4 × 4 × 3 mm3; imaging plane being parallel to the anterior commissure–posterior commissure (AC–PC) image plane. For spatial normalization, a high-resolution T1-weighted anatomical image was also acquired (MPRAGE; TR = 2,000 ms; TE = 2.98 ms; TI = 900 ms; flip angle = 9°; FOV = 256 × 256 mm2; matrix size = 256 × 256; isotropic voxel size = 1 mm).

fMRI data preprocessing. The first five EPI volumes were discarded to allow for signal equilibration. Data preprocessing was performed using Data Processing Assistant for rs-fMRI (DPARSF) [Yan & Zang, 2010], which is based on Statistical Parametric Mapping (SPM8). Image preprocessing comprised of slice timing and head motion correction. The fMRI data were then spatially normalized to the Montreal Neurological Institute (MNI) space with isotropic 3 mm voxel, via the gray matter segment obtained from structural images as follows. Individual T1-weighted image volume was coregistered to the mean fMRI volume, then segmented into gray matter, white matter (WM) and cerebrospinal fluid (CSF) using the New Segment toolbox in SPM8, with custom tissue priors generated from the Template-O-Matic toolbox using the “matched-pair” approach [Wilke, Holland, Altaye, & Gaser, 2008]. Next, we used a diffeomorphic nonlinear registration algorithm (DARTEL) [Ashburner, 2007] to create a study-specific template and to normalize segmented images to the MNI space (with modulation). Individual fMRI volumes were then spatially normalized to the MNI space using the flow fields derived from DARTEL (without modulation), to improve the accuracy of spatial normalization [Tahmasbi, Aboelsamah, Zheng, Munhall, & Johnsrude, 2009]. Spatially normalized fMRI volumes were smoothed with an 8 mm Gaussian kernel. Linear drifts were further removed and the preprocessed EPI data was band-pass filtered (0.01–0.08 Hz).

fMRI data quality control. In-scanner head movements during rs-fMRI would produce spurious results in time series correlation [Power, Barnes, Snyder, Schlaggar, & Petersen, 2012; Satterthwaite et al., 2012; Van Dijk, Sabuncu, & Buckner, 2012]. Participants who exhibited more than 4 time points of excessive motion (>1 mm maximum frame-wise displacement), calculated by the “motion fingerprint” software [Wilke, 2012], in their resting-state scans were excluded from analyses, resulting in exclusion of three boys in the ASD group and two boys in the TD group. There was no time point exhibiting maximum framewise displacement (FD) > 1.5 mm in the EPI data of the participants included in the final analysis. Spatial coverage of normalized EPI data was inspected for each participant, and another five boys with ASD and four TD boys were excluded owing to incomplete coverage of the cerebral cortex. As a result, 40 boys with high-functioning ASD (three with concurrent ADHD) alongside 42 TD boys, with rs-fMRI data of
limited in-scanner head motion and adequate spatial coverage, were included in the final analysis.

**Nuisance signal regression.** Preprocessed fMRI data were further linearly regressed with nuisance covariates, including signals derived from WM, CSF, and 24-autoregressive motion parameters (six realignment motion parameters, six head motion parameters one time point before, and the 12 corresponding squared items) (Friston-24) [Friston, Williams, Howard, Frackowiak, & Turner, 1996]. To demonstrate the robustness of our findings against potential biased group differences introduced by different regression strategies [Gotts, Saad, Jo, Wallace, Cox, & Martin, 2013; Yan et al., 2013], we performed a complementary denoising regression including average global signal as an additional regressor.

**Selection of seeds and functional connectivity analysis.** As defined in prior work [Mars et al., 2012], the seed region of pRTPJ was chosen as a sphere around the center at the MNI coordinate 54, −55, 26, of 4 mm in radius, which has been demonstrated functionally coupled to areas associated with social cognition and the DMN. To investigate the specificity for pRTPJ, we also conducted the same analyses for the posterior part of the left TPJ (pLTPJ, generated by MarsBaR toolbox [Brett, Anton, Valabregue, & Poline, 2002]) as the contralateral homologous Region of interest [ROI] of the pRPTJ, centering at −54, −55, 26. Finally, given the spatial proximity yet functional distinction between pRTPJ and pSTS, we further ensured that the pRTPJ seed did not spatially overlap with right pSTS (MNI coordinate 50, −56, 7; defined based on a meta-analysis by Van Overwalle and Baetens [2009]), as shown in Supporting Information Figure 1.

Whole-brain functional connectivity calculation was performed using the Resting-State fMRI Data Analysis Toolkit (REST) [Song et al., 2011]. The time series within the seed ROI were averaged to generate a reference time series for each participant. Pearson’s correlation was calculated between the seed reference time series and the time series of each single voxel in the whole brain. The individual-level correlational maps were Fisher z-transformed before entering group-level analysis.

**Statistical analysis.** We used SAS version 9.2 (SAS Institute Inc., USA) to conduct data analyses of group comparisons in demographics and clinical measures, and connectivity-behavior correlations. Using SPM8, one-sample t-tests were performed on the z-maps of boys with ASD and TD boys, separately, to show connectivity maps for pRPTJ. Between-group comparison in connectivity of the FPCN was implemented by two-sample t-tests. As suggested by Yan et al. [2013], we included mean FD as a covariate in all group-level analyses to further account for motion artifacts. To explore age-related shift in connectivity as proposed by Uddin, Supekar, and Menon [2013], and the possible effects of IQ, which was not matched between groups, we additionally included age and full-scale IQ as covariates in a subsidiary analysis. Age and full-scale IQ were mean centered across all subjects before modeling.

Voxel-level analyses were restricted in the gray matter region by applying the sample-specific gray matter mask (the threshold of partial-volume-estimate > 0.15). Owing to the finite spatial coverage of the EPI scan, we excluded cerebellum in the analysis by subtracting the cerebellum ROIs derived from the Automated Anatomical Labeling template [Tzourio-Mazoyer et al., 2002] from the gray matter mask. Owing to the denoising model without global signal regression (GSReg) resulting in strong positive correlation, the threshold of the within-group iFC patterns were stringently set at a cluster-wise family-wise-error (FWE)-corrected $P < 0.05$, with a cluster-forming voxel-level threshold of $P < 10^{-10}$. The between-group statistical outcomes were FWE corrected at the cluster-level at $P < 0.05$, and using a cluster forming voxel-level threshold of $P < 0.001$.

To localize areas showing dysconnectivity, and to identify the related Brodmann areas (BA), we used xjView8 toolbox (http://www.alivelearn.net/xjview8/). Stereotaxic coordinates were reported in MNI space. The results were visualized using BrainNet Viewer [Xia, Wang, & He, 2013].

**Network analysis using multiple ROIs.** To further elaborate the relationships between the ROIs and the hub regions within the pRTPJ-associated network in TD and ASD boys, we defined four spherical ROIs of 4 mm in radius, derived from the pRTPJ seed, ventral medial prefrontal cortex (vmPFC), PCC, and the aberrantly connected region (from the aforementioned between-group analysis; see Results). The vmPFC and PCC ROIs were defined according to the peak coordinate in the statistical map from the within-group result in the TD group.

Time series for each of these ROIs were extracted to compute the (Pearson’s) correlation matrix for all ROIs for each participant. Following Fisher’s r-to-z transformation, the differences in correlation coefficients (reflecting the network structure) between each pair of ROIs were compared using independent sample $t$ tests. This group comparison for the correlation coefficients was false discovery rate (FDR)-corrected for multiple comparisons at $q < 0.05$.

**Functional connectivity in relation to social difficulties.** To examine how abnormal iFC with pRTPJ was associated with social difficulties, we used Pearson’s
correlation (r) to demonstrate brain-behavior relationships, as both the behavioral and iFC data were not significantly deviant from normal distribution. Aberrant functional connectivity values were calculated for seed-ROIs pairs, and were transformed to z values. ROIs were defined as a sphere of 4 mm in radius around the peak coordinates within clusters showing significant between-group differences in our main analysis (i.e., without GSReg). Behavioral social deficits were indexed by the total scores of “Qualitative Abnormalities in Reciprocal Social interaction” on the Chinese ADI-R for past behaviors, together with the Chinese SRS (total and subscale raw scores) reflecting current status. FDR correction [Benjamini & Hochberg, 2000] was performed to correct for multiple comparisons, with significance set at $q < 0.05$, and $q$ between 0.05 and 0.1 as trend-level significant.

**Results**

**Demographic and Clinical Characteristics**

Compared to TD boys, boys with high-functioning ASD had lower full-scale and verbal IQs, and higher total scores and subscores of the Chinese SRS. There were no significant group differences in age, handedness and performance IQ. The extent of average FD, based on the measures proposed by either Jenkinson, Bannister, Brady, and Smith [2002] or Power et al. [2012], during rs-fMRI scan was well matched between groups (Table 1 and Supporting Information Fig. 2).

**Right Posterior temporo-parietal Junction was Hyperconnected to Right Ventral Occipito-temporal Cortex in ASD**

TD boys demonstrated pRTPJ iFC with bilateral mPFC, PCC, middle temporal lobe, and contralateral posterior TPJ (Fig. 1), comparable to the known spatial involvement of the DMN [Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010]. Boys with ASD also demonstrated a similar iFC pattern of pRTPJ with the DMN, except for additional involvement of the right ventral occipito-temporal cortex (VOTC).

As shown in Table 2 and Figure 1, boys with ASD, relative to TD boys, had pRTPJ hyperconnectivity with the right VOTC involving the lingual gyrus (LG), fusiform gyrus (FG), and middle occipital gyrus (MOG), each with a group-difference peak. The pRTPJ iFC patterns in both groups and between-group difference were mostly unchanged in the subsidiary analysis including age and full-scale IQ as additional covariates (Supporting Information Fig. 3). There was no significant correlation between age and pRTPJ-right VOTC connectivity (Supporting Information Fig. 4). Similar patterns of group difference were shown after GSReg (Supporting Information Table 1 and Fig. 5). There were no significant group differences in iFC of the pLTPJ in either direction (Supporting Information Fig. 6).

The **Network Structure of pRTPJ Intrinsic Connectivity**

In the correlation matrix of the hubs within the pRTPJ-associated network, we found that the VOTC was only significantly hyperconnected with the pRTPJ, but not with the other components of the network, in boys with ASD relative to TD boys (Table 3 and Fig. 2).

**Correlations between Aberrant iFC of pRTPJ and Social Impairment**

Higher scores in both the ADI-R and the Chinese SRS is indicative of poorer social function. There were significantly positive correlations between the pRTPJ-right MOG connectivity and total SRS scores ($r = 0.35$, $p < 0.01$).

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Table 1. Demographic Features of the Participants

<table>
<thead>
<tr>
<th></th>
<th>ASD ($n = 40$)</th>
<th>TDC ($n = 42$)</th>
<th>t or $\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (range, 9–17 years)</td>
<td>$12.38 \pm 2.17$</td>
<td>$11.64 \pm 2.71$</td>
<td>$t = 1.35$</td>
<td>0.182</td>
</tr>
<tr>
<td>Handedness</td>
<td>Right = 38</td>
<td>Right = 38</td>
<td>$\chi^2 = 0.62$</td>
<td>0.432</td>
</tr>
<tr>
<td>Full-scale IQ</td>
<td>$105.88 \pm 15.14$</td>
<td>$114.00 \pm 11.67$</td>
<td>$t = -2.73$</td>
<td>0.008</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>$105.60 \pm 16.06$</td>
<td>$111.29 \pm 13.45$</td>
<td>$t = -1.74$</td>
<td>0.086</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>$105.75 \pm 15.72$</td>
<td>$114.45 \pm 10.06$</td>
<td>$t = -3.00$</td>
<td>0.004</td>
</tr>
<tr>
<td>ADI-R, social</td>
<td>$17.00 \pm 7.10$</td>
<td>$29.97 \pm 13.42$</td>
<td>$t = 9.68$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SRS, total scores</td>
<td>$88.01 \pm 34.62$</td>
<td>$34.62 \pm 29.97$</td>
<td>$t = 13.42$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Social communication and general features</td>
<td>$37.91 \pm 17.22$</td>
<td>$8.63 \pm 5.18$</td>
<td>$t = 10.06$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Autism mannerism</td>
<td>$18.50 \pm 8.58$</td>
<td>$4.29 \pm 4.01$</td>
<td>$t = 9.28$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Social awareness</td>
<td>$19.48 \pm 6.04$</td>
<td>$13.03 \pm 4.82$</td>
<td>$t = 5.20$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Social emotion</td>
<td>$12.13 \pm 5.31$</td>
<td>$3.89 \pm 3.29$</td>
<td>$t = 8.17$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean of total frame-wise displacement, Jenkinson* (mm)</td>
<td>$0.136 \pm 0.038$</td>
<td>$0.133 \pm 0.060$</td>
<td>$t = 0.23$</td>
<td>0.821</td>
</tr>
<tr>
<td>Mean of total frame-wise displacement, Power* (mm)</td>
<td>$0.234 \pm 0.010$</td>
<td>$0.231 \pm 0.016$</td>
<td>$t = 0.13$</td>
<td>0.899</td>
</tr>
</tbody>
</table>

*Mean of total frame-wise displacement was calculated based on Jenkinson et al. [2002] and Power et al. [2012].

Abbreviations: ASD = autism spectrum disorder; TDC = typically developing control; ADI-R = Autism Diagnostic Interview-Revised; SRS = Social Responsiveness Scale.
and the “social communication” \( (r = 0.38, q = 0.044) \), the “social emotion” \( (r = 0.36, q = 0.044) \) and the “autism mannerism” \( (r = 0.36, q = 0.044) \) sub-scores. pRTPJ connectivity with other two subregions showed trends of positive correlations with the same SRS scores \( (r = 0.24–0.30, q = 0.075–0.083) \). There was also a significantly positive correlation between social reciprocity deficits on the ADI-R and pRTPJ-right FG connectivity \( (r = 0.35, q = 0.044) \). In the TD group, in contrast, no significant correlations were found between iFC and social functioning indexed by the SRS. The group differences in correlation patterns; however,
did not reach statistical significance when using a multiple regression model with iFC as the dependent variable and group, SRS, and group-by-SRS interaction as regressors, to test the effect of group-by-SRS interaction on iFC (Table 4, Fig. 3, and Supporting Information Figs. 7 and 8).

Discussion

In this study, we found that pRTPJ showed adult-like resting-state connectivity with brain regions associated with the DMN in both TD and ASD boys. However, boys with ASD showed hyperconnectivity between the pRTPJ and VOTC compared to TD boys, which was associated with the severity of their social deficits. Our findings provide empirical support for “dysconnectivity” as a neurobiological characteristic associated with ASD, which is further associated with social deficits, an essential feature of ASD.

iFC of pRTPJ and the Relation to DMN

The pRTPJ has been reported distinctively engaged in mentalizing [Decety & Lamm, 2007; Kubt & Jack, 2013], and functionally coupled with the dorsal medial prefrontal subsystem of the DMN [Andrews-Hanna et al., 2010] in mediating introspection about mental status [Andrews-Hanna, 2012]. Our findings that the posterior parts of bilateral TPJ were temporally synchronized with PCC, mPFC, and middle temporal lobe, spatially comparable to the DMN in adulthood in both groups [Andrews-Hanna et al., 2010], suggest that specific functional connection of the TPJ [Mars et al., 2012] could also be observed in late childhood, even in those with ASD. This supports the concept that functional brain organization is by and large conserved throughout the developmental process [Supekar, Musen, & Menon, 2009; Uddin, Supekar, Ryali, & Menon, 2011].

Hyperconnectivity between the pRTPJ and VOTC in ASD

The hyperconnected cluster with the pRTPJ in ASD, when compared with TD, involves several regions in the right VOTC, including the right LG, right MOG, and right FG. Multiple regions of VOTC have been reported as key nodes of the visual recognition network responding to a range of visual stimuli [Price & Devlin, 2011]. Specifically, the LG is implicated in deriving biological forms from motion information in TD individuals [Materna, Dicke, & Thier, 2008; Pelphrey, Morris, & McCarthy, 2005; Servos, Osu, Santi, & Kawato, 2002]. The MOG acts together with LG in processing biological motion information [Dayan, Casile, Levit-Binnun, Giese, Hendler, & Flash, 2007; Pelphrey, Morris, Michelich, Allison, & McCarthy, 2005], and joins forces with the posterior part of the FG for scenes/objects recognition [de Haan & Cowey, 2011; Saygin, Osher, Koldewyn, Reynolds, Gabrieli, & Saxe, 2012; Sowards, 2011]. Regarding FG, notably, the spatial extent of the aberrant cluster we found spans from the right mid-FG posteriorly to the MOG. This area is posterior-medial to, and may be functionally distinct from, the fusiform face area [Caspers, Zilles, Eickhoff, Schleicher, Mohlberg, & Amunts, 2013; Dalton et al., 2005; Kanwisher & Yovel, 2006; Saygin et al., 2012], which has been reported atypical in mediating facial processing and attribution of social meaning in individuals with ASD [Dichter, 2012]. This midposterior part of the FG is necessary for physical aspects of facial perception [Kanwisher & Yovel, 2006; Peelen & Downing, 2005], processing geometric information of actions [Dayan et al., 2007] and implicit emotional processing [Sabatnelli et al., 2011], and may demonstrate an activation pattern with gradient of increasing object selectivity and decreasing face selectivity from the mid-FG to the MOG [Caspers et al., 2013; Weiner & Grill-Spector, 2010]. Acting coordinately, the posterior-FG influences the MOG, then to the LG during processing biological
motion information [McKay, Simmons, McAleer, Marjoram, Piggot, & Pollick, 2012]. Taken together, the atypically connected right VOTC cluster observed in this study may be implicated in nonfacial visual processing. Meta-analyses based on activation likelihood estimation have revealed under-activation of the mid-FG yet over-activation of the posterior-FG during social tasks in ASD [Di Martino, Ross, Uddin, Sklar, Castellanos, & Milham, 2009; Lombardo, Baron-Cohen, Belmonte, & Chakrabarti, 2011]. Individuals with ASD also display enhanced task-related activity for a broad range of visual functioning in the low-level visual cortices overlapping with the aberrant connected cluster found here [Samson, Mottron, Soulieres, & Zeffiro, 2012]. Some studies [Ecker et al., 2012; Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2006] suggest that neuroanatomical abnormalities in the occipito-temporal cortices might partially contribute to social impairment in ASD. However, it remains unclear how interactions between lower-level sensory-perceptual processing cortices and regions mediating higher-level cognition contribute to the development of ASD. Our study, therefore, shed new light on the role of brain regions involving in nonface visual processing and its aberrant connection to the mentalizing network in ASD.

In terms of connectivity, there have been reports that the VOTC is functionally coupled with the task-positive network [Grady et al., 2010; Kennedy & Courchesne, 2008], and has functional connections with the pSTS during both rest and task conditions [McKay et al., 2012; Shih, Keuhn, Oram, Leyden, Keown, & Muller, 2011; Turk-Browne, Norman-Haignere, & McCarthy, 2010]. The pSTS is implicated in representing and identifying biological motion [Hein & Knight, 2008], perceiving intentions [Vander Wyk, Hudac, Carter, Sobel, & Pelphrey, 2009], and facial processing [Hein & Knight, 2008]. Interactions between the VOTC and the pSTS may be essential for integrating information from faces and actions with the social/physical contexts to facilitate the understanding of intention. These external social cues may be processed from the VOTC to pSTS, then relayed to the anatomically adjacent pRTPJ, which is a critical junction area where different types of social information are assembled and integrated [Kubit & Jack, 2013] (as illustrated in Supporting Information Fig. 1). The integration of these lower-level processes contributes to higher-level mentalizing, for which rTPJ is directly involved [Saxe & Kanwisher, 2003]. Therefore, our findings of increased connectivity between the VOTC and pRTPJ in boys with ASD may reflect interference to the integration of incoming sensory information important for social cognition, resulting in social difficulties in ASD.

**Figure 3.** Exploratory correlations between aberrant connectivity and degree of social deficits in boys with ASD. Scatter plots show the associations between the iFC ($r_z$; $z$-transformed correlational value) between the pRTPJ and right middle occipital gyrus (rMOG) and (A) the Chinese version of SRS total scores, (B) the SRS “communication” subscores, (C) the SRS “autism mannerism” subscores, and (D) the SRS “social emotion” subscores. (E) Scatter plot shows the association between the pRTPJ-right fusiform gyrus (rFG) iFC and the Chinese version of ADI-R social scores. Other scatter plots for trend-level correlations between atypical iFC and autistic symptom measures are provided in Supporting Information Figures 4 and 5.
Our findings of the hyperconnectivity between pRTPJ and right VOTC is in contrast to the prediction from an overall underconnectivity in ASD [Just et al., 2012; Muller et al., 2011; Vissers et al., 2012]. Nonetheless, emerging literature now reports a mixed pattern of under- and over-connectivity in individuals with ASD [Di Martino et al., 2014; Fishman et al., 2014]. It remains unclear whether intrinsic hyperconnectivity in ASD can be explained in terms of developmental shift [Uddin, Supekar, & Menon, 2013], compensatory strategies [Kana et al., 2011], or differences in methodological approaches [Nair, Keown, Datko, Shih, Keehn, & M. Menon, 2011], or differences in methodological approaches [Uddin, Supekar, Holtmann, Freitag, & Poustka, 2011; Constantino & Gruber, 2005]. A speculation is that hyperconnectivity might restrain adaptable resource allocation, resulting in mental inflexibility and insistence for sameness. Alternatively, this finding may be explained by the fact that some behaviors related to social-communication domain in the original SRS [Constantino & Gruber, 2005], for example, “turn-taking interactions,” “starting social interactions,” are now categorized in the “autism mannerism” subdomain by factor analysis in the Chinese SRS [Gau et al., 2013]. This specification would indicate that the “autism mannerism” subscores, though named so, still indexes inflexibility of social reciprocity to a certain extent.

Interestingly, we did not observe significant association between the connection aberrancy and “social awareness” scores of the Chinese SRS. In terms of psychological constructs, “social awareness” involves multiple components, including perception, self-other recognition, interpretation, and memory [Wegner & Giuliano, 1983]. Lumpning together heterogeneous behaviors within the “social awareness” domain might hinder the detection of small-effect correlations. Another reason for this lack of detectable correlation may be of psychometric reasons. The Chinese ADI-R is an investigator-based semistructured interview scale for parents of individuals with ASD to assess their child’s core autistic features [Gau et al., 2010; Rutter et al., 2003], whereas the Chinese SRS is a parent-report questionnaire about the extent of autistic-like traits of the child [Gau et al., 2013]. In light of this, the noted moderate correlation between SRS and ADI-R [Bolte, Westerwald, Holtmann, Freitag, & Poustka, 2011; Constantino et al., 2003] echoes the observed differences in the associations of atypical iFC with the severity of social difficulties indexed by different scales. Nonetheless, we observed the same directionality of brain-behavioral associations across the ADI-R and SRS scores, substantiating the validity of the findings. Notably, although an optimized FDR approach was used to control for type I error, we should acknowledge the caveat to interpret the trend-level associations. Future study with a larger sample size and better indices for social cognition may be of detectable correlation may be of psychometric reasons. The Chinese ADI-R is an investigator-based semistructured interview scale for parents of individuals with ASD to assess their child’s core autistic features [Gau et al., 2010; Rutter et al., 2003], whereas the Chinese SRS is a parent-report questionnaire about the extent of autistic-like traits of the child [Gau et al., 2013]. In light of this, the noted moderate correlation between SRS and ADI-R [Bolte, Westerwald, Holtmann, Freitag, & Poustka, 2011; Constantino et al., 2003] echoes the observed differences in the associations of atypical iFC with the severity of social difficulties indexed by different scales. Nonetheless, we observed the same directionality of brain-behavioral associations across the ADI-R and SRS scores, substantiating the validity of the findings. Notably, although an optimized FDR approach was used to control for type I error, we should acknowledge the caveat to interpret the trend-level associations. Future study with a larger sample size and better indices for social cognition will be helpful.

In this study, only the pRTPJ seed displayed atypical iFC in the ASD group. Notably, the atypically

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**Table 4. Correlations between Autistic Social Difficulties and iFC for the Two Groups**

<table>
<thead>
<tr>
<th></th>
<th>pRTPJ-rLingual</th>
<th>pRTPJ+rMOG</th>
<th>pRTPJ+rFG</th>
<th>r</th>
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<tbody>
<tr>
<td>Autism spectrum disorder</td>
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<td>ADI-R, social</td>
<td>0.05 0.343</td>
<td>0.15 0.195</td>
<td>0.35 0.044*</td>
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<tr>
<td>SRS, total scores</td>
<td>0.25 0.083*</td>
<td>0.35 0.044*</td>
<td>0.27 0.075*</td>
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<tr>
<td>Social communication</td>
<td>0.29 0.075*</td>
<td>0.38 0.044*</td>
<td>0.30 0.075*</td>
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<tr>
<td>Autism mannerism</td>
<td>0.25 0.083*</td>
<td>0.36 0.044*</td>
<td>0.27 0.076*</td>
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<tr>
<td>Social awareness</td>
<td>0.05 0.343</td>
<td>0.08 0.304</td>
<td>0.16 0.189</td>
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<tr>
<td>Social emotion</td>
<td>0.24 0.083*</td>
<td>0.36 0.044*</td>
<td>0.27 0.076*</td>
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<td>Typically Developing Controls</td>
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<tr>
<td>SRS, total scores</td>
<td>−0.02 1</td>
<td>−0.11 1</td>
<td>−0.08 1</td>
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<tr>
<td>Social communication</td>
<td>0.13 1</td>
<td>−0.03 1</td>
<td>0.09 1</td>
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<tr>
<td>Autism mannerism</td>
<td>0.04 1</td>
<td>−0.04 1</td>
<td>−0.03 1</td>
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<tr>
<td>Social awareness</td>
<td>−0.24 0.895</td>
<td>−0.22 0.895</td>
<td>−0.33 0.69</td>
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<tr>
<td>Social emotion</td>
<td>0.03 1</td>
<td>−0.03 1</td>
<td>0.00 1</td>
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*Significant correlation between social difficulties and functional connectivity (g < 0.05, FDR corrected).

* Marginally significant correlation between social difficulties and functional connectivity.

Abbreviations: ADI-R = Autism Diagnostic Interview-Revised; SRS = Social Responsiveness Scale; pRTPJ = right posterior temporoparietal junction; rLingual = right lingual gyrus; rMOG = right middle occipital gyrus; rFG = right fusiform gyrus.
connected VOTC was found in the right hemisphere. Some studies [Kanwisher, McDermott, & Chun, 1997; Peelen & Downing, 2005; Pelphrey, Morris, & McCarthy, 2005] suggest a right lateralized system of occipital-temporal regions engaged in the sensory processing of socially relevant information, while regions involved in social communication, including PCC and mPFC, are left-lateralized [Gotts, Jo, Wallace, Saad, Cox, & Martin, 2013]. This suggests the quintessence in integration of bilateral hemispheric activity for complex social cognition. However, we did not identify significant group differences in iFC of the posterior TPJ on the left hemisphere, even though pLTPJ has been suggested to play a role in social-communication [Ciaraldi, doro et al., 2007; Saxe & Kanwisher, 2003]. A formal examination of group differences in the laterality of iFC is, however, beyond the scope of this study. Better-powered datasets are needed to formally examine hemispheric differences in iFC of TPJ, to further test for atypical asymmetry of neural networks in ASD [Cardinale, Shih, Fishman, Ford, & Muller, 2013].

Methodological Considerations

To prevent potential bias in the observed group differences introduced by GSReg [Gotts, Saad et al., 2013; Saad et al., 2012], regression model without GSReg was opted as the main denoising method. However, this strategy might risk inadequately accounting for confounds from motion artifacts [Power, Mitra, Laumann, Snyder, Schlaggar, & Petersen, 2014; Yan et al., 2013]. We, therefore, additionally examined the potential effect of GSReg, and the results endorse robustness of the main findings.

We applied several strategies to address the concerns about in-scanner head motion impacts on iFC. These include stringent motion inclusion criteria, matching the ASD and control groups on average FD, nuisance regression against Friston-24 parameters at an individual level, together with individual mean FD included as a covariate in the group-level analysis [Yan et al., 2013]. The combination of these strategies is expected to minimize the impacts of head-motion on iFC studies.

To avoid exhausting visual stimulation, the participants were asked to keep their eyes closed throughout rs-fMRI image acquisition. Prior studies focusing on the impact of eye-open/closed states on iFC differences reported that functional connectivity maps of DMN [Yan et al., 2009] and all major neural networks [Patriat et al., 2013] appear to be similar across eye-open/closed conditions. BOLD signal intensity [Feige, Scheffler, Esposito, Di Salle, Hennig, & Seifritz, 2005] and test-retest reliability [Patriat et al., 2013] of the visual network are higher for eye-open when compared to eye-closed conditions. However, these works were done with TD participants, and it is unknown if the effects of eye-opening vs. closed are dependent on the presence of neuropsychiatric conditions. Future studies should focus on the impacts of eye-open/closed conditions in relation to current findings in an ASD population, given that there is still a lack of consensus in this aspect in acquiring rs-fMRI data.

Limitations

Several limitations should be acknowledged. First, as the participants are of average or above-average IQ and are biologically male, it is, thus, unknown whether the current findings could be generalized to other subgroups on the spectrum, given the heterogeneity in ASD [Lai, Lombardo, Chakrabarti, & Baron-Cohen, 2013]. Second, the study is cross-sectional and correlational, thus cannot address etiological and developmental effects. Third, three participants with co-occurring ASD and ADHD were not excluded in the current study, rendering possible confounding comorbidity effects. However, a high co-occurring rate of ADHD in ASD has been reported [Taurines, Schwenck, Westerwald, Sachse, Siniatchkin, & Freitag, 2012], and partly common causal factors are likely shared by the two conditions [Rommelse, Geurts, Franke, Buitelaar, & Hartman, 2011]. Future works investigating individuals with ASD with/without ADHD will help address the issue. Lastly, given the moderate-sized sample of this study, the findings await to be replicated in independent datasets in the future.

Our data provide new evidence that intrinsic hyperconnectivity between the pRTPJ and the right VOTC was associated with social deficits in boys with high-functioning ASD. Considering that pRTPJ is a key structure for human social cognition, and that the right VOTC is essential for processing socially related visual information, we speculate that atypical pRTPJ-right VOTC connection may hinder effective integration of socially relevant perceptual information, contributing to social difficulties in ASD. Our findings provide empirical support for understanding the neurobiology of ASD in the framework of “dysconnectivity”, rather than uniformly under-connected neural networks.

Acknowledgments

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References


**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.