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Sensory-to-Cognitive Systems Integration Is Associated With Clinical Severity in Autism Spectrum Disorder

RH = Sensation-to-Cognition Integration in ASD

Kenia Martínez, PhD, Magdalena Martínez-García, BS, Luis Marcos-Vidal, BS, Joost Janssen, PhD, Francisco X. Castellanos, MD, Clara Pretus, PhD, Óscar Villarroja, MD, Laura Pina-Camacho, MD, Covadonga M. Díaz-Caneja, MD, Mara Parellada, MD, Celso Arango, MD, Manuel Desco, MD, Jorge Sepulcre, MD, and Susanna Carmona, PhD

Dr. Martínez and Ms. Martínez-García are co-first authors of this article.

Drs. Sepulcre and Carmona are co-last authors of this article.

Editorial

Supplemental Material

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ABSTRACT

Objective: Impaired multisensory integration in autism spectrum disorder (ASD) may arise from functional dysconnectivity among brain systems. Our study examines the functional connectivity integration between primary modal sensory regions and heteromodal processing cortex in ASD, and whether abnormalities in network integration relate to clinical severity.

Method: We studied a sample of 55 high-functioning ASD and 64 healthy controls (HC) male children and adolescents (total n = 119, age range 7-18). Stepwise functional connectivity analysis (SFC) was applied to resting state functional magnetic resonance images (rsfMRI) to characterize the connectivity paths that link primary sensory cortices to higher-order brain cognitive functional circuits and relate alterations in functional connectivity integration with three clinical scales: Social Communication Questionnaire, Social Responsiveness Scale, and Vineland Adaptive Behavior Scales.

Results: HC displayed typical functional connectivity transitions from primary sensory systems to association areas, but the ASD group showed altered patterns of multimodal sensory integration to heteromodal systems. Specifically, compared to the HC group, the ASD group showed (1) hyper-connectivity in visual cortex at initial link step distances; (2) hyper-connectivity between sensory unimodal regions and regions of the default mode network; and (3) hypo-connectivity between sensory unimodal regions and areas of the fronto-parietal and attentional networks. These patterns of hyper- and hypo-connectivity were associated with increased clinical severity in ASD.

Conclusion: Network-wise reorganization in high-functioning ASD individuals affects strategic regions of unimodal-to-heteromodal cortical integration predicting clinical severity. Additionally, SFC analysis appears to be a promising approach for studying the neural pathophysiology of multisensory integration deficits in ASD.

Key words: Autism spectrum disorder, resting-state functional magnetic resonance imaging, multisensory integration, fronto-parietal networks, default mode network

INTRODUCTION

Successful integration of sensory inputs is crucial for both basic perceptual functions and higher-order processes related to social behavior and cognition. Autism spectrum disorder (ASD) is characterized by impairments in social communication and language acquisition, restricted interests and repetitive behaviors.¹ Altered sensory and perceptual processes are common in individuals with ASD.² Over 95% of children with ASD display hyper- and hypo-sensitivities in primary perceptual domains.²⁻⁴ These unimodal alterations are accompanied by an impaired ability to integrate sensory information at the multimodal level. Impairments at different hierarchical processing steps may cascade into the hallmark higher-order social and cognitive deficits characteristic of ASD⁵⁻⁷.

Individuals diagnosed with ASD, when compared to healthy controls (HC), have shown brain architectural alterations in the form of (1) long-distance functional hypo-connectivity, particularly between frontal and posterior cortical regions⁸ and (2) local hyper-connectivity mainly within posterior regions, including primary sensory cortices^{9,10}. Nevertheless, the difficulty individuals with ASD have in integrating inputs from multiple sensory modalities is proposed to stem from altered hierarchical patterns of cerebral organization rather than from local or distributed individual systems¹¹⁻¹³. Alterations in hierarchical patterns of cerebral organization are challenging to detect mainly due to the limited number of network analytical methods explicitly designed to evaluate network integration (rather than network segregation) of brain systems. As a result, functional connectivity deficits in ASD have primarily been evaluated with non-

integrative connectivity approaches¹⁴, precluding knowledge about how multiple brain systems assemble together in individuals with ASD.

In this study, we used an analytical integrative method called step-wise functional connectivity (SFC).^{15,16} This method combines traditional seed-based and graph theory approaches to assess the hierarchical integration taking place from unimodal sensory regions (visual, auditory and somatosensory) to heteromodal regions.

To our knowledge, this is the first study applying SFC to explore hierarchical brain organization in young people diagnosed with ASD. We hypothesized that (1) different relay stations within the functional connectivity pathways from primary sensory cortices to higher-order cognitive circuits would be altered in ASD compared to HC; and (2) these altered integration connectivity patterns in individuals with ASD would be associated with increased clinical severity.

3. METHOD

3.1. Sample Description

We examined a subsample of the aggregated multi-site dataset provided by the ABIDE I (http://fcon_1000.projects.nitrc.org/indi/abide/abide_I.html) and ABIDE II (http://fcon_1000.projects.nitrc.org/indi/abide/abide_II.html) initiatives. The ABIDE I repository consists of 1112 data sets including 539 patients with ASD and 573 HC. ABIDE II was established to further promote discovery science on the brain connectome in ASD. The ABIDE II repository consists of 1114 data sets including 521 patients with ASD and 593 HC with greater phenotypic characterization, particularly in regard to measures of core ASD and associated symptoms. All data were fully anonymized in accordance with HIPAA (Health Insurance Portability and

Accountability) guidelines, and research procedures and ethical guidelines were followed in accordance with local Institutional Review Boards (IRB).

To avoid variability related to data acquisition site, we only included individuals from the NYU Langone Medical Center, the site with the largest sample size of individuals who fulfilled the following inclusion criteria: (1) male sex; (2) age up to 18 years old; (3) predominantly right-handed (Edinburgh Handedness Inventory score > 0); (4) estimated full scale intelligence quotient (IQ) score higher than 80 per the four-subtest Wechsler Abbreviated Scale of Intelligence (WASI); and (5) mean frame-wise displacement below 0.2 mm.

A total of 55 individuals with high-functioning ASD (31 of ABIDE I and 24 of ABIDE II initiatives) and 64 HC (41 of ABIDE I and 23 of ABIDE II initiatives) met the inclusion criteria. Table 1 includes the demographic and clinical characteristics of the final sample. The ABIDE IDs of the subjects are provided in Table S1, available online. When taking together both datasets, the frame-wise displacement range was .03 - .2 mm (Mean_{ASD} = .08; Mean_{HC} = .06; $t = 4$; $p < .001$).

 INSERT TABLE 1 ABOUT HERE

A detailed description of site-specific protocols of diagnostic criteria, ethical procedures, clinical assessment, inclusion criteria and magnetic resonance imaging (MRI) acquisition parameters are available at the ABIDE website (http://fcon_1000.projects.nitrc.org/indi/abide/abide_I.html). Note that NYU Langone Medical Center participated in ABIDE I and ABIDE II initiatives using the same phenotypic characterization and scan parameters. The ASD group from NYU Langone Medical Center dataset included individuals with *DSM-IV-TR* diagnoses of Autistic

Disorder, Asperger's Disorder, or Pervasive Developmental Disorder Not-Otherwise-Specified. This diagnosis was based on a review of participant's history, the Autism Diagnostic Observation Schedule,¹⁷ and the Autism Diagnostic Interview-Revised¹⁸ when possible. The Schedule of Affective Disorders and Schizophrenia for Children-Present and Lifetime Version (KSADS-PL)¹⁹ was applied to assess psychopathology for differential diagnosis and to determine comorbidity with Axis-I disorders. Subjects with Manic or Depressive episode, Bipolar Disorder, Schizophrenia, or Post-traumatic Stress Disorder within 3 months prior to the evaluation were excluded from the ASD group. Selection criteria for control individuals were mainly based on the absence of any current Axis-I disorder. For both groups, current chronic systemic medical conditions, contraindications to MRI scanning and use of antipsychotics were exclusionary.²⁰ Written informed consent/assent was obtained for all participants.

The following questionnaires provided indices of symptomatic severity and adaptive functioning: Social Communication Questionnaire (SCQ),²¹ Social Responsiveness Scale (SRS)²², and Vineland Adaptive Behavior Scales-Second Edition (VABS)²³. SCQ is a parent questionnaire with 40 yes-or-no items that assesses the communication skills and social functioning of an individual who may have ASD. The SRS is a parent or teacher questionnaire (65 items on a 4-point Likert scale) that identifies social impairment associated with ASD and quantifies its severity. The VABS is a structured interview that takes approximately 20-60 minutes used to assess adaptive behaviors, including four broad domain areas: communicative, motor, daily living and social skills. The VABS was administered to parents for all children (< 18 years of age). For the current study we used SCQ and SRS total scores and the ABC score derived from the VABS. Higher scores in both SCQ and SRS, and lower scores in VABS, indicate greater symptom severity and worse adaptive behavior.

3.2 Image Acquisition and Preprocessing

MRI data were acquired using a 3 Tesla Siemens Allegra scanner. Details regarding acquisition parameters are provided in the ABIDE website (http://fcon_1000.projects.nitrc.org/indi/abide/scan_params/NYU/). Briefly, a 6-minute rsfMRI scan sequence was acquired using a multiecho-planar imaging (EPI) sequence. Multiecho parameters were as follows: TR = 2.0 s, TE = 33ms, FA = 90°, matrix size = 30 x 80 x 80, voxel size = 3 x 3 x 4 mm. In addition, a high-resolution 3D T1-weighted image was obtained for anatomic reference.

Images were processed at the Neuroimaging Compute Facilities (Center for Brain Science, Harvard University) using the standard pipeline for rsfMRI data. Preprocessing steps included (1) removal of the first four volumes to allow for T2-equilibration; (2) slice timing correction; (3) motion correction; (4) normalization to MNI152 standard space at 4 mm isotropic voxel resolution; (5) regressing out nuisance covariates, which included head motion parameters, global mean, white matter, and cerebrospinal fluid signals; (6) band-pass filtering the time-series data ($.009 \text{ Hz} < f < .08 \text{ Hz}$) to reduce low-frequency drift and high-frequency physiological noise; and (7) spatial smoothing with a 6 mm full-width-at-half-maximum (FWHM) Gaussian kernel. Finally, the rsfMRI data were down-sampled to 8 mm isotropic voxels to reduce computational demands^{15,24}. A pipeline with the main image preprocessing steps is depicted in Figure 1A.

 INSERT FIGURE 1 ABOUT HERE

3.3. Combined Stepwise Functional Connectivity Analysis

The combined SFC analysis computes the degree of functional connectivity from three unimodal sensory seed regions simultaneously at different link-step distances, to obtain a combined map of all sensory functional streams^{15,16}. Figure 1 (B and C) summarizes the main analytical steps for computing SFC degree. First, the time courses in the preprocessed and normalized BOLD images were correlated between pairs of voxels to obtain individual weighted connectivity matrices (Figure 1B). Second, the matrices were filtered to include only positive correlations due to the ambiguous interpretation of negative correlations²⁵. Next, a stringent false discovery rate (FDR) correction ($q < .001$) was applied to the p-values of the correlation matrix to control for the rate of false-positive connections²⁶. Finally, the FDR-corrected connectivity matrices were binarized and submitted to SFC analysis.

To calculate the combined SFC maps, three primary sensory seeds - corresponding to the visual, auditory and somatosensory primary cortices- were defined as cubic regions of eight voxels each (Figure 1C). The MNI coordinates (x, y, z) for the most lateral, anterior, and superior voxels for each seed were as follows: [8, -76, 10] for the visual seed, [56, -12, 10] for the auditory seed, and [0, -28, 66] for the somatosensory seed. Next, the SFC degree of every single voxel-node at each link-step distance was computed as the sum of pathways connecting that particular voxel-node with the unimodal seed regions. Each link-step distance was defined as the number of edges that pertain to a path connecting a given node to the seed regions. Thus, a path is defined as a sequence of linked nodes that never visit a single node more than once. As demonstrated in previous studies^{15,27}, functional pathways ‘collapse’ in regions previously described as cortical hubs for link-step distances above seven. Therefore, a distance of seven steps was set as the optimal number of link-step distances. Additionally, we confirmed that correlations between SFC degree maps reach the

maximum value (i.e., 1) at link step seven in both the ASD and HC groups (see Figure S1, available online). The SFC values were standardized at each link-step to normalize the effect of escalating probability of connections with seeds as the number of steps increases. The standardized value per subject n , node i and link-step j , was calculated according to the formula:

$$zSFCdegree_{nij} = \frac{SFCdegree_{nij} - \text{mean}(SFCdegree_{nj})}{\text{standarddeviation}(SFCdegree_{nj})}$$

Therefore, each map represents the relative increase of SFC degree across different link-step distances. Finally, $zSFCdegree$ maps were converted into images for statistical analysis.

3.4. Statistical Analysis

One-sample and two-sample t-tests were computed in MATLAB R2015a (<https://es.mathworks.com>) using SPM12 (<http://www.fil.ion.ucl.ac.uk/spm>). The MATLAB function 'glmfit.m' was used to perform correlation analyses with clinical scales. Figure 1D schematically illustrates the statistical analysis workflow.

3.4.1. Characterization of SFC pathways in ASD and HC groups

One-sample t-tests were performed to characterize the SFC patterns in the HC and ASD groups separately. Only FDR-corrected voxels ($q < .0001$) were considered as significant. Frame-wise displacement and age were set as covariates.

3.4.2. Group differences in SFC degree

Two-sample t-tests were calculated to examine diagnostic differences in SFC degree at each of the seven link-step distances. Frame-wise displacement and age were set as covariates. Since no clusters survived the FDR correction used in the one-sample

t-tests ($q < .0001$), Monte-Carlo simulation based on cluster size was implemented to correct for multiple comparisons

[https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html]. Only clusters exceeding 1024 mm^3 at the voxel-wise threshold (i.e., probability of a type-I error) of .005 were considered significant, resulting in a corrected experiment-wise error rate of .05.

Finally, a bootstrap sampling technique was implemented in MATLAB (R2017b) for validating these latter findings in 1000 simulated datasets ($n = 119$; with replacement) randomly selected from the original sample. The bootstrap method is a flexible and powerful statistical tool that can be used to quantify the uncertainty associated with a given estimator. This method allows to assess the stability of the results by estimating the standard error of the estimator (in this case, the t-values for the group differences), accounting for the distortions in the findings due to sample specificities²⁸. Mean, standard error and confidence interval ($\alpha = .95$) for the t-values computed in 1000 simulated samples at each link step distance were obtained. Mean d-values of the distributions were reported only for the voxels that significantly differed between ASD and HC in the 95% of the samples.

3.4.3. Association between altered SFC degree and clinical variables in ASD

Regression analyses were performed within the ASD group to examine the relationship between SFC degree and clinical severity after regressing out the effects of frame-wise displacement and age. Analyses were computed on each region where the ASD and HC groups differed significantly in the two-sample t-tests (Section 2.5.2). First, mean SFC degree values for each significant cluster at each link-step were computed from each individual SFC degree map. In the linear model, each of the

clinical scales (i.e., SRS, SCQ and VABS) was set as the independent variable one at a time, and frame-wise displacement and age were added as covariates. Next, t-values for the clinical scale effects were converted to Pearson correlation coefficients according to the formula (df = degrees of freedom):

$$r = \sqrt{\frac{t^2}{(t^2 + df)}}$$

3.5. Cortical Visualization

Caret software was used to visualize the results from one-sample and two-sample t-tests. Statistical maps corrected for multiple comparisons were projected onto right and left cerebral hemispheres of the PALS surface (PALS-B12) using the ‘enclosing algorithm’ and ‘multifiducial mapping’ settings²⁹.

4. RESULTS

4.1. Stepwise functional connectivity maps in HC and ASD

Figure 2 shows the right hemisphere SFC maps for the HC and ASD groups (Figure S2 includes left hemispheres SFC maps. See Supplemental Material, available online). Overall, HC and ASD displayed similar connectivity streams at early link-step distances. At one and two link-step distances both groups showed functional connectivity among unimodal sensory systems. However, there was a qualitative difference at the third link-step distance between the statistical maps computed for the ASD and HC groups. Compared to the HC group, the ASD group showed lower number of voxels with significant functional connectivity streams between sensory regions and some multimodal integration areas such as the Lateral Occipitotemporal Junction (LOTJ) and dorsolateral prefrontal cortex (DLPF).

From four to seven link-step distances, sensory functional streams for both the HC and ASD groups connected to higher-order cognitive regions (cortical hubs), such

as fronto-parietal executive network nodes (i.e., dorsolateral prefrontal and inferior parietal cortex) and default mode network (DMN) nodes (i.e., precuneus, medial prefrontal cortex, lateral temporal cortex and temporo-parietal junction).

 INSERT FIGURE 2 ABOUT HERE

4.2. Atypical SFC degree in ASD compared to HC along the sensation-to-cognition continuum

Figures 3 shows the between-group differences between the ASD and HC groups in SFC degree. A mixed pattern of hypo- and hyper-connectivity was found in the ASD group compared to the HC group. At link-step distance one – corresponding to classic seed-based functional connectivity analysis – the ASD group showed decreased SFC degree between the sensory seeds and frontal, parietal and temporal regions spatially overlapping with fronto-parietal and attentional networks. A similar pattern was observed at longer link-steps distances, as well as hypo-connectivity between the sensory seeds and somatosensory-motor/ superior parietal regions.

 INSERT FIGURE 3 ABOUT HERE

Conversely, increased SFC degree was found between the three sensory seeds and several regions, overlapping with visual, default-mode and limbic networks in individuals with ASD compared to controls. The functional hyper-connectivity pattern between the sensory seeds and the DMN involved the bilateral precuneus, posterior cingulate gyrus, bilateral angular gyrus, bilateral superior frontal.

Only one cluster overlapping with the frontoparietal – ventral attention networks exhibited hyper-connectivity at longer link step distances. Of note, this cluster, located in the middle frontal gyrus, was hypo-connected with the sensory seeds at earlier link step distances.

4.3 Association between atypical SFC and clinical severity in ASD

Figures 4A present the regions showing between-group differences in SFC degree (two-sample t-tests) that significantly correlated with SRS, SCQ and VABS scales in the ASD group. Note that higher scores in both SCQ and SRS, and lower scores in VABS, indicate increased severity of symptoms and worse adaptive behavior. Across all the link-step distances and the different clinical scales, most of the significant correlations fitted the following ‘rule’: for those regions where patients with ASD had abnormally higher SFC degree compared to controls, increased SFC degree was associated with greater clinical severity. Accordingly, for those regions where patients with ASD had abnormally lower SFC degree relative to HC, increased SFC degree correlated with poorer adaptive behavior and decreased SFC degree correlated with better adaptive behavior.

As an example of the above-mentioned pattern, Figure 4B shows the scatter plots for the significant correlations at link-step distance three. As observed, SFC degree was associated with clinical scores in two clusters. Following the aforementioned ‘rule’: VABS scores were positively correlated with SFC degree in the right superior frontal gyrus, a cluster showing decreased SFC degree in patients diagnosed with ASD compared to HC individuals. Conversely, VABS scores were negatively associated with SFC degree in the left angular gyrus, which showed increased SFC degree in ASD compared to controls.

INSERT FIGURE 4 ABOUT HERE

Only two clusters fitted the opposite pattern, the left middle frontal at step distance one and the right inferior parietal-supramarginal gyrus at longer step distances. Note that these two clusters correspond to regions of the attentional and frontoparietal networks, and the cluster in the middle frontal gyrus was hypo-connected with the sensory seeds at earlier link step distances.

5. DISCUSSION

In the current study we used stepwise functional connectivity analysis to characterize the connectivity paths that link primary sensory cortices to higher-order brain cognitive functional circuits in ASD and evaluate whether abnormalities in network integration relate to clinical severity. We found atypical functional transitions from unimodal to multimodal cortical areas in high-functioning male children and adolescents with ASD compared to HC individuals. Specifically, abnormal functional hypo-connectivity in ASD was mainly observed in parietal and frontal regions of the attentional and frontoparietal networks, while significantly increased hyper-connectivity in ASD was detected in regions overlapping the visual cortex and the DMN. Importantly, our findings of hyper- and hypo-connectivity were associated with clinical severity within children and adolescents diagnosed with ASD.

ASD has been characterized by dysfunctional patterns of brain connectivity. Conventional seed-based, as well as more sophisticated network-based and/or graph theory strategies, have shown evidence that the ASD phenotype is associated with a mixed pattern of hypo-connectivity and hyper-connectivity. Our results support these

previous findings and also add key information about how cerebral systems are reorganized and reintegrated in the brain of individuals with ASD. Functional connectivity at the shortest link distance, which is equivalent to traditional seed-based analysis, reflects (1) a direct functional hyper-connectivity between unimodal sensory seeds and cortical regions spatially overlapping with the DMN (left angular gyrus, bilateral precuneus and bilateral posterior cingulate) and the visual network; and (2) functional hypo-connectivity between unimodal sensory seeds and areas of the frontoparietal and attentional networks. Moreover, our analytical approach allows for analysis of indirect functional connectivity (medium and large connectivity distances from the seed), which is thought to provide information about hierarchical flow across specific brain networks. Our results indicate that the pattern of hyper- and hypo connectivity in high-functioning ASD is observed along the sensation-to-cognition continuum. We also found that regions showing hypoconnectivities at shorter link step distances were hyperconnected at larger link step distances, which is the case of the cluster in the left middle frontal gyrus.

A rather consistent finding from functional studies has been decreased connectivity between anterior-posterior areas, whose coordination is critical in accomplishing many higher-order cognitive functions^{8, 30-32}. Local connectivity intensification at the primary cortices in ASD has been proposed to generate delays in the perceptual pipeline and information transmission to subsequent systems, compromising the integration of information^{11,33}. Thus, integration deficits may impair earlier stages of information processing, with cascading effects on higher-order social and cognitive functions leading to the deficits observed in ASD. Conversely, in our study we found those deficits in the sensation-to-cognition continuum. Sensory unimodal seeds were hypo-connected to multimodal parietal and prefrontal integration

regions overlapping with fronto-parietal and ventral-dorsal attention networks. At the same time, a pattern of hyper-connectivity in high-functioning ASD was also observed in the visual cortex, which is consistent with the predisposition for local processing in ASD^{34,35}, potentially at the expense of global gestalt processing³⁶.

We also document a hyper-connectivity pattern along the sensation-to-cognition continuum, between unimodal seeds and regions spatially overlapping with the DMN. The DMN is extensively associated with ‘Theory of Mind’ (ToM), defined as the ability to attribute mental states to others³⁷; and ToM deficits are considered a hallmark feature of ASD. The DMN is also involved in introspective thought and self-reflection when subjects are alert but not actively involved in an externally oriented or goal-directed task^{38,39}. In contrast, a temporally separable “task-positive” network (TPN), including the dorsolateral prefrontal cortex (DLPF), parietal cortex, and Supplemental motor area, is involved in cognitive tasks that require attention to the external environment³⁹. We observed that primary sensory regions were hyper-connected to several DMN regions, such as precuneus-posterior cingulate, angular gyrus and medial prefrontal cortex, and also hypo-connected with ventral and dorsal attention regions. These results could imply that the switch between externally oriented attention-demanding tasks and internally oriented thoughts is abnormal in ASD, which is consistent with studies reporting less task-induced deactivation of the DMN in ASD³¹.

Finally, correlational analysis with clinical scales suggests that the pattern of hyper-and hypo-connectivity found in ASD patients may be useful for predicting clinical severity. Specifically, we found that increased clinical severity was associated with (1) hyper-connectivity between unimodal seeds and DMN regions (i.e., the angular gyrus), and (2) hypo-connectivity between unimodal sensory systems and multimodal integration regions (i.e., superior frontal, superior parietal). These findings are coherent

with previous studies reporting a positive relationship between hyper-connectivity in posterior brain regions and symptom severity as measured by social reciprocity and repetitive behavior scores from Autism Diagnostic Observation Schedule (ADOS) ⁴⁰. Also, higher SRS scores in ASD patients were reported to be associated with (1) an abnormal interplay between primary somatosensory-motor networks and other networks ¹⁰; and (2) less task-induced deactivation of the DMN ³¹. Taken together, these results support the view that impairments in the ability to successfully integrate multisensory information could negatively impact social functioning in ASD ⁴¹.

The limitations of this study mainly concern to the restrictive sample selection criteria we used, which resulted in a relatively homogeneous sample of patients with ASD. Accordingly, results may not generalize to other subtypes of ASD (e.g., those with IQ < 80 or female patients). Another consequence of restrictive selection criteria is that sample size was reduced, decreasing sensitivity to detect small but meaningful effects.

In summary, we used SFC analysis to examine functional pathways across brain systems as a proxy of how information flows are differentially propagated in the brain of persons diagnosed with ASD. We describe the network-wise reorganization of unimodal-to-heteromodal cortical integration in high-functioning male children and adolescents with ASD, from which we derive three main conclusions: (1) disordered functional connectivity between unimodal systems (first steps) and multimodal integration regions (intermediate steps) in ASD may affect the hierarchical nature of multisensory integration from modular sensory brain areas up to integrative association nodes; (2) atypical hyper-connectivity between unimodal primary cortices and DMN regions could imply an aberrant interaction between externally and internally oriented

processing systems in ASD; and (3) altered SFC may be a promising marker for impaired multimodal sensory information processing and socialization deficits in ASD.

ACCEPTED MANUSCRIPT

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Table 1. Demographic and Clinical Characteristics

Variables	HC			ASD			Statistics
	n	Mean	SD	n	Mean	SD	(Two-sample t-tests)
Initiatives							
ABIDE I	41			31			
ABIDE II	23			24			
DSM-IV-TR Diagnostic Category							
Autism				24			
Asperger				28			
PDD-NOS				3			
Age (range 7 to 18)	64	10.64	2.81	55	11.71	2.9	t = -2.011 (p = .047)
Medication status							
Taking medication	NA			16			
Not taking medication	NA			39			
Co-morbidity							
ADHD	0			22			
MD-NOS	0			7			
GAD	0			3			
Specific Phobia	0			2			
Social Phobia	0			1			
IQ							
Full Scale	64	113.94	13.72	55	109.05	16.24	t = -1.78 (p = .07)
Verbal	64	114.34	13.29	55	106.18	14.3	t = -3.22 (p = .002)
Performance	64	110.44	15.12	55	111	18.02	t = .18 (p = .85)
Clinical Scales							
SRS [Total]	62	23.76	13.54	54	89.68	29.22	t = 15.92 (p < .0001)
SCQ [Total]	52	3.04	2.37	50	15.9	6.94	t = 11.44 (p < .0001)
VABS [ABC Standard]	46	104.72	12.19	43	83.98	10.73	t = -8.5 (p < .0001)

Note. ADHD = attention-deficit/hyperactivity disorder; ASD = autism spectrum disorder; GAD = Generalized Anxiety Disorder; HC = healthy controls; MD-NOS = Mood disorder not otherwise specified; PDD-NOS = Pervasive Developmental Disorder Not-Otherwise-Specified; SCQ = Social Communication Questionnaire²¹; SRS = Social Responsiveness Scale²²; VABS = Vineland Adaptive Behavior Scale [ABC Standard: Adaptive Behavior Composite Standard]²³.

Figure 1. Main Analytical Steps for Computing Stepwise Functional Connectivity Degree and Statistical Analysis Workflow

Note: Summary of the steps for (A) rsfMRI image preprocessing; (B and C) stepwise functional connectivity (SFC) analysis; and (D) statistical analyses to test hypotheses. ASD = autism spectrum disorder; HC = healthy controls.

Figure 2. Stepwise Functional Connectivity Maps in Healthy Controls and Autism Spectrum Disorder (One Sample *t*-Tests)

Note: (A) Stepwise functional connectivity maps (right hemisphere) in high-functioning male children and adolescents diagnosed with autism spectrum disorder and healthy control individuals. In the normalized color scale, 0 represents non-significant results ($q < .0001$, FDR corrected) and 1 is the maximum *d* value corresponding to the smallest *p*-value. (B) Initial (binarized average of the one step and two steps maps) intermediate (binarized average of the three to five steps maps) and terminal (binarized average of the six and seven steps maps) cores for healthy adults (adapted from ¹⁵). Labels in A: AI = Anterior Insula; dACC = Dorsal Anterior Cingulate Cortex; DLPF = Dorsolateral Prefrontal Cortex; FEF = frontal eye field; LOTJ = Lateral Occipitotemporal Junction; OP = Operculum Parietale; PCS = Posterior Central Sulcus; SMA = Supplemental Motor Area; SPC=Superior Parietal Cortex; VPMC = Ventral Premotor Cortex.

Figure 3. Atypical Functional Connectivity Degree in Autism Spectrum Disorder Compared to Healthy Controls Along the Sensation-To-Cognition Continuum

Note: (A) Group differences between high-functioning male children and adolescents diagnosed with autism spectrum disorder (ASD) and healthy control (HC) individuals in stepwise functional connectivity (SFC) degree. In the maps are reported the mean *d*-

values for the voxels that significantly differed between ASD and HC in the 95% of the 1000 simulated datasets ($n = 119$; with replacement) randomly selected from the original sample. (B) Seven large-scale resting-state functional networks⁴².

Figure 4. Association Between Atypical Functional Connectivity Degree and Clinical Severity in Autism Spectrum Disorder

Note: (A) Clusters where altered stepwise functional connectivity degree in the autism spectrum disorder (ASD) group significantly correlates with clinical scales [Social Communication Questionnaire (SCQ), Social Responsiveness Scale (SRS) and Vineland Adaptive Behavior Scales (VABS)]. Note that lower scores in SRS and SCQ and higher scores VABS indicate better adaptive behavior. Colored cells correspond to hypo-connectivity (blue) and hyper-connectivity (yellow) in high-functioning male children and adolescents diagnosed with ASD compared to healthy control (HC) individuals. (B) Scatter plots for the correlations between clinical scales and stepwise functional connectivity (SFC) degree at link-step distance three.

A. Image Preprocessing

- (1) Removal of the first four volumes for T2-equilibration
- (2) Slice timing correction
- (3) Motion correction
- (4) Normalization to MNI152 (voxel size: 4 x 4 x 4 mm)
- (5) Regressing out nuisance covariates
- (6) Band-pass filtering (0.009 Hz < f < 0.08 Hz)
- (7) Spatial smoothing (6 mm FWHM)
- (8) Down-sampling the images (voxel size: 8 x 8 x 8 mm)

B. Computation of binary functional connectivity matrices **C. Computation of Stepwise Functional Connectivity (SFC) Degree** **D. Statistical Analyses**

A. Stepwise Functional Connectivity [One-sample t-tests]

B. From Sensation to Cortical Hubs: Characteristic Initial, Intermediate and Terminal Cores

A. Healthy Controls vs. Autism Spectrum Disorder [Two-samples t-tests]

[Significant group differences in 1000 replication samples (confidence interval 95%)]

B. Resting State Networks

A. Correlations with clinical scales

Link-Step Distance	Peak location (x, y, z) [Anatomical Region]	Resting State Network (Yeo et al., 2011)	SRS		SCQ		VABS	
			r	p	r	p	r	p
1	-38,42,32 (Left Middle Frontal Gyrus)	Ventral Attention/ FrontoParietal	0.21	0.13	0.33	0.02	-0.30	0.06
2	-22,-94,16 (Left Superior Occipital Gyrus)	Visual	0.29	0.04	0.10	0.48	0.09	0.58
3	10,10,64 (Right Superior Frontal Gyrus)	Ventral Attention/ Somatosensory-Motor	-0.17	0.23	-0.21	0.16	0.32	0.04
3	-50,-70,40 (Left Angular Gyrus)	Default Mode	-0.02	0.87	0.10	0.51	-0.31	0.05
4	10,2,72 (Right Superior Frontal Gyrus)	Ventral Attention/ Somatosensory-Motor	-0.16	0.25	-0.33	0.02	0.23	0.14
4	50,-54,64 [Right Inferior Parietal]	FrontoParietal	-0.08	0.60	0.37	0.01	0.24	0.12
5	10,2,72 (Right Superior Frontal Gyrus)	Ventral Attention/ Somatosensory-Motor	-0.20	0.16	-0.29	0.04	0.14	0.38
5	50,-54,64 [Right Inferior Parietal]	FrontoParietal	-0.04	0.80	-0.35	0.01	0.24	0.13
6	-38,-38,64 (Left Postcentral Gyrus - Superior Parietal)	Dorsal Attention/ Somatosensory-Motor	-0.26	0.06	-0.23	0.11	0.32	0.04
6	50,-54,64 [Right Inferior Parietal]	FrontoParietal	-0.02	0.87	-0.32	0.03	0.23	0.14
6	10,2,72 (Right Superior Frontal Gyrus)	Ventral Attention/ Somatosensory-Motor	-0.16	0.25	-0.32	0.03	0.24	0.13
7	-38,-38,64 (Left Postcentral Gyrus - Superior Parietal)	Dorsal Attention/ Somatosensory-Motor	-0.27	0.05	-0.24	0.11	0.32	0.04

ASD > TD
 TD > ASD

The higher the score the worse the clinical profile
The higher the score the better the clinical profile

B. Correlations with clinical scales at link step distance three