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ORIGINAL PAPER

Characterization of Autism Spectrum Disorder across the Age Span by Intrinsic Network Patterns

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Abstract

Autism spectrum disorder (ASD) is characterized by abnormal functional organization of brain networks, which may underlie the cognitive and social impairments observed in affected individuals. The present study characterizes unique intrinsic connectivity within- and between- neural networks in children through to adults with ASD, relative to controls. Resting state fMRI data were analyzed in 204 subjects, 102 with ASD and 102 age- and sex-matched controls (ages 7–40 years), acquired on a single scanner. ASD was assessed using the autism diagnostic observation schedule (ADOS). BOLD correlations were calculated between 47 regions of interest, spanning seven resting state brain networks. Partial least squares (PLS) analyses evaluated the association between connectivity patterns and ASD diagnosis as well as ASD severity scores. PLS demonstrated dissociable connectivity patterns in those with ASD, relative to controls. Similar patterns were observed in the whole cohort and in a subgroup analysis of subjects under 18 years of age. Greater inter-network connectivity was seen in ASD with greater intra-network connectivity in controls. In conclusion, stronger inter-network and weaker intra-network resting state-fMRI BOLD correlations characterize ASD and may differentiate control and ASD cohorts. These findings are relevant to understanding ASD as a disruption of network topology.

Keywords Autism spectrum disorder · fMRI · brain · Multivariate analysis · Child · Adult

Introduction

Autism spectrum disorder (ASD) is characterized by deficits in social and cognitive function. While the hallmarks of the ASD are social and communication deficits, as well as stereotyped or repetitive behaviors, children often demonstrate comorbid difficulties in intelligence, memory, hyperactivity and learning (Myers, Johnson, & American Academy of Pediatrics Council on Children With Disabilities, 2007). The recent impetus to devise effective therapeutic strategies for affected children is constrained by limited understanding of the neurological substrates of these impairments.

The study of large-scale brain networks may elucidate novel markers to facilitate the evaluation of the efficacy of various treatment strategies for ASD. One particular imaging modality, resting-state functional magnetic resonance imaging (fMRI), has advanced our understanding of ASD, yet much of the published literature is difficult to interpret, and occasionally, contradictory. Early studies suggested that brain networks may be both abnormally hyper- and hypo-connected in ASD (Kana et al. 2011). Some authors proposed that long-range connections are weaker in ASD relative to typically developing (TD) controls (Vissers et al. 2012), while others have suggested a link between local hyper-connectivity in posterior regions and ASD symptom severity (Keown et al. 2013). Recent reviews have highlighted the variability across studies and their various

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approaches that could account for the inconsistent findings (Loomes et al. 2017; Nair et al. 2014; Vasa et al. 2016).

One general consensus from the mixed literature is that ASD is less a disorder of over- and/or under-connectivity, but rather, one characterized by 'disrupted cortical connectivity' (Kana et al. 2011). ASD has also been described as an 'idiosyncratic disorder' (Hahamy et al. 2015), whereby individuals with ASD show more variability in network connectivity strengths compared to TD individuals. fMRI studies have increasingly investigated whether differences between typical development and ASD can be explained by abnormal organization in specific brain networks. Various resting-state networks, demonstrating increased connectivity at rest, are well-established in the literature (Brier et al. 2012; de Pasquale et al. 2012) and have been characterized in developmental populations (Ibrahim et al. 2014; Schäfer et al. 2014).

Further, specific resting-state networks have been associated with the atypical phenotype observed in children with ASD. The best studied of these is the default mode network (DMN, see Padmanabhan et al. 2017, for a review). Strengths within nodes of the DMN inversely correlated with ASD symptom severity scores (Assaf et al. 2010; Washington et al. 2014), while the absence of normative developmental increases in strength within the DMN has been noted in children with ASD (Washington et al. 2014). The posterior cingulate cortex, a major hub of the DMN, was more strongly connected to regions outside the DMN in individuals with ASD compared to controls (Doyle-Thomas et al. 2015). A triple network model of psychopathology suggests that atypical interactions between the DMN and SAL may contribute to a lack of engagement with socially-relevant stimuli (Menon 2011). Studies of other networks have shown that functional segregation of regions in the pre-central gyrus were associated with both ASD severity and social-communicative skills (Nebel et al. 2014), while Uddin et al. 2013 reported that connectivity strengths within nodes of the salience network (SAL) were related to restricted and repetitive behaviours in children with ASD. ASD is also characterized by deficits in language, communication and social interactions. Previous studies have shown abnormal neural function within the language network (LAN) (Verly et al. 2014a, b). Connectivity between the dorsal attention network (DAN) and the DMN has been associated with restrctive behaviors in children at risk of developing ASD in resting state data (McKinnon et al. 2018). The authors hypothesize that inverse functional relationships between DAN and DMN networks are part of typical development and that atypical hyperconnectivity between the DMN and DAN at rest may play a role in the limited cognitive flexibility seen in ASD. Furthermore, the aforementioned study noted these same stereoyped behaviors were associated with connectivity between the visual network (VIS) and the DMN. In a study involving children with high-functioning ASD, decreased functional connectivity between the ventral attention network (VAN) and salience network was found at resting state immediately after performing a working memory task (Bernas et al. 2018). The authors hypothesized that this described a failure of bridging of emotoinal states, whereas children with ASD exhibtied a more rigid system in terms of emotional-executive bridging and may relate to the attention and emotional deficits seen in ASD. Finally, abnormalities within the motor network (MOT) in resting state data have also been reported in ASD (Carper et al. 2015; Floris et al. 2016; Nebel et al. 2014, 2016), which may explain the motor deficits commonly found in ASD. Taken together, these studies demonstrate that within- and between-network connectivity may be important contributors to ASD, and highlight the importance of studying connectivity across multiple networks, including the DMN, VAN, DAN, VIS, MOT, LAN and SAL networks.

Questions regarding network connectivity patterns can be explored using advanced tools, including graph theory (Rubinov and Sporns 2010), independent component analysis (ICA) (Beckmann et al. 2004) and partial least squares (PLS) (McIntosh and Lobaugh 2004). Recent reports have included multivariate approaches to determine connectivity profile differences between those with and without ASD. Chen et al. (2015) examined fMRI in adolescents, data collected across six different sites (from the ABIDE data set), and found atypical connectivity between the frontoparietal, DMN and cingulo-operculum networks, with the latter two related to social/communication deficits. Multivariate analyses of resting state fMRI have also been applied to other neurodevelopmental disorders, focussing on anterior and posterior cingulate cortex and BOLD signal variability (Zöller et al. 2018, 2017).

Given the converging findings that ASD is (i) a condition characterized by dysfunctional circuitry within individual resting-state networks; (ii) associated with altered relations between distinct networks; and (iii) is a dynamic developmental process, progressing through childhood and adulthood, we performed a multivariate analysis of resting-state networks to identify associations between imaging data and clinical phenotypes. The goals of the present study were first to determine whether global connectivity patterns within and across networks were differentially expressed between ASD and TD groups, second, whether these evolved with age and third, whether these patterns could explain significant variance in ASD symptom severity. To do, so we employed a multivariate analysis approach, partial least squares (PLS) (McIntosh and Lobaugh 2004)—a data-driven method that can characterize global connectivity patterns. PLS decomposes the covariance matrix between independent and dependent variables, producing orthogonal components that represent the association between these sets of variables. In this case, the independent variables were fMRI connectivity measures, and the dependent variables were group (ASD vs TD) and ASD severity. One advantage to PLS is that because there is only 1 decomposition computation, we are not concerned with multiple comparisons, a common issue in conventional correlation-based analyses. In addition, our decomposition is not affected by collinear variables, which typically complicates GLM-based analyses of this type. PLS allows us to find a pattern that spans across all of our ROIs that is associated with our dependent variable of interest, and permutation testing and bootstrap resampling can be used to determine significance of that pattern, as well as the significant contributors to that pattern (McIntosh and Lobaugh 2004). We structured our functional connectivity analysis by network to gain a better understanding of large-scale connectivity patterns and interactions associated with ASD across seven of the classic functional networks from early childhood to mid-adulthood. Our study extends previous work, partially owing to our large study sample from a single site and scanner, that our ASD and controls groups are carefully matched to balance covariates and minimize bias from confounding effects and examining a set of networks within the same multivariate model.

Methods

Participants

After screening for acceptable head motion (process described below), fMRI resting state data were analyzed from 102 subjects with ASD between the ages of 7 and 40 years. Three analyses were performed. First, from a cohort of 247 TD subjects between the ages of 7 and 40, nearest neighbour, 1-to-1 propensity matching (Ho et al. 2011) was performed to select 102 TD participants who were matched to the ASD group based on age and sex. Second, a follow-up analysis including only subjects under 18 years of age was conducted. Third, an analysis including only subjects with ASD who had an ADOS severity score (N = 85) was performed to explore behavioural correlates with connectivity. All data were selected from a large cohort of participants from studies at the Hospital for Sick Children. Exclusion criteria for all participants included the presence of any neurological disorders, medical illness, prematurity, uncorrected vision, IQ < 70 as determined by the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 2002), as well as standard MRI contraindicators (e.g., ferromagnetic implants). A history of developmental delay, learning disability and attention deficit hyperactivity disorder (ADHD) and other psychiatric disorders was used to exclude TD children; ADHD and other psychiatric comorbidities were not primary diagnoses in any of the participants with ASD. This study was approved by the Research Ethics Board at the Hospital for Sick Children. All children provided informed assent and parents and adult participants provided informed written consent. See Table 1 for a summary of study demographics; age distributions for participants under-18 are provided in Fig. 1 as an example of matched age distributions.

Behavioural Measures

Diagnosis of ASD was confirmed based on clinical evaluation, previous diagnostic reports and/or the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 2000; Rutter et al. 2012). The ADOS is a semi-structured standardized interview that assesses autism symptoms in the domains of communication, reciprocal social interaction, restricted interests, repetitive behaviour and imagination and creativity, with higher scores indicative of greater severity of ASD symptoms. The clinical threshold (i.e., diagnostic criteria) for ASD is a total score of ≥ 7, and average total score fell above this threshold in the current study ($M = 10.9$, $SD = 3.8$). 83% of participants with ASD had complete ADOS scores that could be converted to severity scores ($M = 6.3$, $SD = 2.4$) (Gotham et al. 2009; Hus and Lord 2014). Severity scores facilitate comparability between different modules of the ADOS and serve as an autism severity index that accounts for age and language proficiency, with a

Table 1 Demographic, IQ and motion information of each analysis

			TD			ASD		
Analysis	N (TD:ASD)	Sex (M:F)	Mean age (SD)	Mean IQ (SD)	Mean motion (SD)	Mean age (SD)	Mean IQ (SD)	Mean motion (SD)
1) ASD-TD 7–40	102:102	78:24	17.2 (7.9)	114.1 (SD)	0.22 (0.18)	17.3 (8.7)	103.6 (SD)	0.29 (0.21)
2) ASD-TD 7–18	71:71	58:13	12.4 (2.4)	112.7 (SD)	0.26 (0.20)	12.3 (2.4)	100.1 (SD)	0.32 (0.22)
3) ASD severity	0:85	64:21	–	–	–	16.0 (8.0)	104.1 (16.3)	0.29 (0.21)

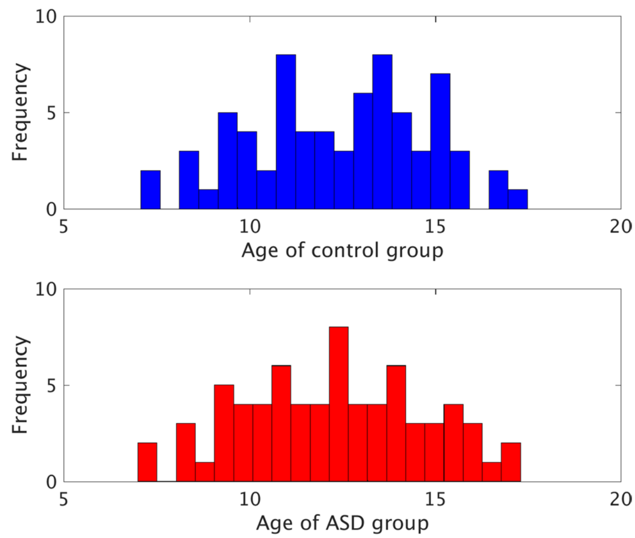

Fig. 1 Propensity-matched age distributions for the analysis of subjects under 18 years of age

score of ten being the most severe. Our sample covered the full range of severity scores (1–10), with a mean of 6.3 and standard deviation of 2.4.

MRI Protocol and Preprocessing

MRI data were collected using a 3T MRI (Tim Trio, Siemens) and a 12-channel head coil at the Hospital for Sick Children in Toronto, Canada. Head stabilization was achieved with foam padding. The scanning protocol included an anatomical T1-MPRAGE (1 mm iso; TR/TE/TI/FA = 2300/2.96/900/9) and a 4.68 min EPI resting state fMRI (3.5 mm iso; TR/TE/FA = 2340/30/70; 120 TRs). During the fMRI sequence, participants were asked to fixate on a cross within a circle displayed via an MR-compatible goggle system. fMRI preprocessing steps were performed using standard AFNI (Cox 1996) and FSL tools (Jenkinson et al. 2012), and included slice-timing and motion correction, spatial smoothing (7 mm kernel) and bandpass temporal filtering (0.01–0.1 Hz). Whole brain average signal and max-displacement (MD) motion signal were regressed from the data. The MD signal was found using *3dvolreg*, which represents the maximum Euclidean distance travelled by any voxel within the brain and is calculated using the 6-parameter rigid body transformation matrix computed from each volume to a reference volume. Stringent motion criteria were met for inclusion in the study. Head motion during the fMRI sequence was deemed acceptable if greater than 3/4 of the volumes were less than 2 mm from the median head position. Participants were not included in the study if these criteria were not met. Mean distance from the median head position was calculated for each subject and was used as a metric to compare motion between groups (Table 1).

Connectivity Matrix Calculation

Following preprocessing, connectivity matrices were calculated between 47 regions of interest (ROIs). These ROIs represent seven brain networks: dorsal attention, ventral attention, default mode, visual, motor, language and salience networks. Except for the salience network, which was derived from coordinates reported by Brier et al. (2012), all node coordinates were derived from Pasquale et al. (2000). Spherical ROIs (5 mm radius) centred on the coordinates from these studies, were registered into each subject's individual fMRI space using a two-step registration including the subject's anatomical T1. ROI names and coordinates are listed in Table 2.

PLS Analysis

PLS was used to explore connectivity patterns across all seven networks simultaneously that may differentiate TD and ASD groups, as well as patterns associated with ASD severity. PLS is a multivariate method for finding an association between two sets of variables. In this case, one set of variables was the connectivity matrix of fMRI BOLD correlations, while the other was group (ASD vs. TD) or ASD severity score. PLS is performed by calculating the covariance between the two sets of variables, then decomposing the resulting covariance matrix through singular value decomposition. The open-access PLS MATLAB toolbox from the Rotman Research Institute was used to perform these calculations (McIntosh and Lobaugh 2004). For differentiation between TD and ASD groups, the mean-centring method within the PLS toolbox was used. For ASD severity associations, the behaviour method in the PLS toolbox was used. Permutation testing and bootstrap resampling were used to determine statistical significance of connectivity patterns (components) and contributions of individual nodes to these patterns, respectively. Bootstrapping allows for the calculation of bootstrap ratios for each element (connection between two ROIs) of the connectivity matrix, where each ratio approximates a z-score for that element's contribution to the association of interest. Therefore, connections which significantly contribute to group differentiation and variance in ASD severity scores may be determined using standard z-thresholds.

Results

Motion Considerations

Mean head motion for each group and analysis is provided in Table 1. No significant difference was found in mean MD

Table 2 ROI information ((de Pasquale et al. 2012), (Brier et al. 2012))

Index #	Region	Abbreviation	Network	X (mm)	Y (mm)	Z (mm)
1	Left posterior intra parietal sulcus	LpIPS	DAN	−25	−67	48
2	Right posterior intra parietal sulcus	RpIPS	DAN	23	−69	49
3	Left frontal eye field	LFEF	DAN	−26	−12	53
4	Right frontal eye field	RFEF	DAN	30	−13	53
5	Left middle temporal	LMT	DAN	−43	−72	−8
6	Right middle temporal	RMT	DAN	42	−70	−11
7	Right middle frontal gyrus	RMFG	VAN	41	17	31
8	Right pre-central sulcus	RPCS	VAN	41	2	50
9	Right supramarginal gyrus	RSMG	VAN	52	−48	28
10	Right superior temporal gyrus	RSTG	VAN	58	−48	10
11	Right ventral frontal cortex	RVFC	VAN	40	21	−4
12	Left angular gyrus	LAG	DMN	−43	−76	35
13	Right angular gyrus	RAG	DMN	51	−64	32
14	Posterior cingulate/precuneus	PCC	DMN	−3	−54	31
15	Ventral medial prefrontal cortex	vMPFC	DMN	−2	51	2
16	Dorsal medial prefrontal cortex	dMPFC	DMN	−13	52	23
17	Right medial prefrontal cortex	RMPFC	DMN	2	53	24
18	Left inferior temporal gyrus	LITG	DMN	−57	−25	−17
19	Left area v1	LV1	VIS	−3	−101	−1
20	Right area v1	RV1	VIS	11	−88	−4
21	Left area v2 dorsal	LV2d	VIS	−8	−99	7
22	Right area v2 dorsal	RV2d	VIS	14	−96	13
23	Left area v3	LV3	VIS	−9	−96	13
24	Right area v3	RV3	VIS	20	−95	18
25	Left area v4	LV4	VIS	−31	−77	−17
26	Right area v4	RV4	VIS	27	−71	-14
27	Left area v7	LV7	VIS	−23	−78	26
28	Right area v7	RV7	VIS	32	−78	25
29	Left second somatosensory	LSII	MOT	−60	−28	24
30	Right central sulcus	RCS	MOT	35	−26	55
31	Left central sulcus	LCS	MOT	−37	−19	53
32	Right second somatosensory	RSII	MOT	57	−28	23
33	Left supplementary motor area	LSMA	MOT	−1	−17	55
34	Right supplementary motor area	RSMA	MOT	4	−15	53
35	Left putamen	LPUT	MOT	30	−18	10
36	Right putamen	RPUT	MOT	30	−17	9
37	Left dorsal inferior frontal gyrus	LDIFG	LAN	−44	23	15
38	Superior temporal sulcus	STS	LAN	−50	−54	22
39	Anterior superior temporal gyrus	T1a	LAN	−56	−12	−3
40	Upper part of pars opercularis of inferior frontal gyrus	F3OPD	LAN	−44	21	24
41	Pars triangularis opercularis of inferior frontal gyrus	F3TV	LAN	−43	20	4
42	Posterior superior temporal gyrus	T1p	LAN	−55	−48	15
43	Right anterior cingulate cortex	rPGACC	SAL	12	32	30
44	Left anterior cingulate cortex	lPGACC	SAL	−13	34	16
45	Right ventral anterior cingulate cortex	rSGACC	SAL	10	34	16
46	Left insula	Lins	SAL	−42	6	4
47	Right insula	Rins	SAL	43	7	2

motion between ASD and TD groups in the under-18 analysis ($p=0.10$, *t*-test). A mean group difference was found in the 7–40 year-old analysis ($p=0.02$, *t*-test). However, the mean distance from the median head position is very low in each group (< 0.3 mm), strict inclusion criteria for head motion was in place, and motion parameters were regressed from the BOLD timeseries of each subject; thus, this group difference was unlikely to bias our analysis. In the ASD symptom severity analysis, head motion was not correlated with severity score ($p=0.91$, Pearson correlation).

Connectivity Patterns Differentiating TD and ASD Groups

Two analyses were performed to explore the connectivity patterns differentiating TD and ASD groups. A summary of statistical outputs from all analyses is provided in Table 3. First, all subjects, 7–40 years of age, were included. PLS analysis computed a significant component ($p=0.008$, permutation testing) that included several significant bootstrap ratios ($|Z|>1.96$), demonstrating dissociable connectivity patterns in those with ASD, relative to matched controls (Fig. 2a, left panel). The order of elements within each of the labelled networks in the matrix can be found in Table 2. The entire pattern of highlighted connections (which correspond to resting state fMRI temporal correlations) represent the group differentiation. Only significant contributions are plotted, as thresholded by their corresponding bootstrap ratios. An averaged 7×7 network-level matrix was constructed and thresholded to display only the upper and lower 10% of the component contributions (right column of Fig. 2a). In the network level matrices, diagonal elements represent within network connections, while the remaining elements represent between-network connections. Positive contributions (yellow) represent connections that were stronger within or between networks in those with ASD, and negative contributions (blue) represent connections that were stronger in controls. As illustrated by the blue squares along the diagonal, stronger intra-network connections were found in controls in the ventral attention, default mode and MOT. As illustrated by the yellow squares off the diagonal, for the subjects with ASD, greater inter-network connections were seen between the MOT and the default mode and ventral attention networks.

Second, an analysis including only subjects under 18 years of age was performed to explore whether findings would be consistent without the inclusion of adults. PLS computed a significant component differentiating the ASD and TD groups ($p=0.016$, permutation testing) with several significant contributions ($|Z|>1.96$, bootstrap resampling. Similar to the first analysis, the corresponding pattern is illustrated in Fig. 2b. In TD children, the same pattern of greater intra-network connections as the previous analysis is observed, including the ventral attention, default mode and MOT. However, the TD child-adolescent group also exhibited increased inter-network connectivity between the ventral attention and language networks. Again, children with ASD showed greater inter-network connectivity between the motor and default mode networks, but also between the VIS and the language and default mode networks.

Associations of Connectivity with ASD Severity Score

Using PLS, we computed a component that included several significant bootstrap ratios ($|Z|>1.96$, bootstrap resampling), demonstrating associations between ASD symptom severity and within-network connectivity in the salience network. However, the component itself was not significant (permutation testing yielded a p-value of $p=0.23$) and therefore not shown.

Age Associations with PLS Scores

To investigate the effect of age on the above findings, the "brain score" (McIntosh and Mišić 2013; McIntosh and Lobaugh 2004) of each subject was plotted with their age. These represent the degree to which each subject expresses the group difference (Fig. 2) (McIntosh and Mišić 2013). The scores-by-age plots for each analysis in Fig. 2 are provided in Fig. 3. No significant correlations between age and scores were found for either group in any analysis (columns 4 and 5 of Table 3). Further, an analysis of covariance (ANCOVA) was performed to test for a group-by-age interaction of scores and no significant interactions were found (column 3 of Table 3).

Table 3 Summary of PLS findings for each analysis

Analysis	PLS p-value	Age interaction	Age correlation-TD	Age correlation-ASD
1) ASD-TD 7–18	0.016	$p=0.16$	$p=0.27$	$p=0.35$
2) ASD-TD 7–40	0.008	$p=0.94$	$p=0.10$	$p=0.17$
3) ASD severity	0.238	–	–	$p=0.52$

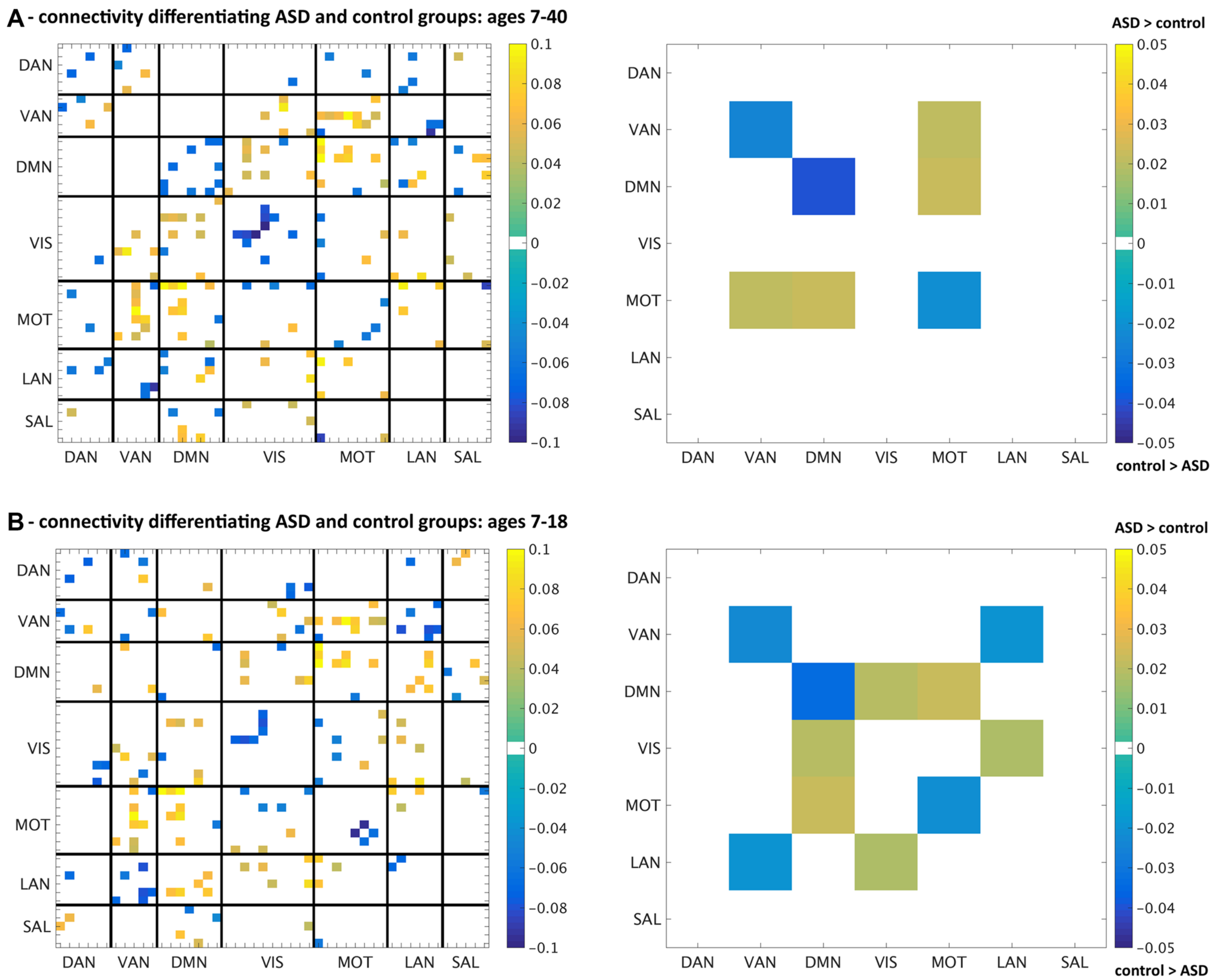

Fig. 2 **a** Thresholded connectivity patterns differentiating subjects with and without ASD between 7 and 40 years of age; **b** thresholded connectivity patterns differentiating children with and without ASD between 7 and 18 years of age. On the right, the connectivity is averaged by network (±top 10%). Networks include: dorsal attention (DAN), ventral attention (VAN), default mode (DMN), visual (VIS), motor control (MOT), language (LAN) and salience (SAL) networks

Discussion

The literature involving neural network connectivity in children with ASD is inconsistent, with conflicting evidence of hypo- and hyper-connectivity. Here, we sought to study the association between connectivity within- and between-intrinsic connectivity networks and the ASD phenotype. The current study provides a global network-based view of altered functional connectivity in ASD by identifying widespread differences in inter- and intra-network connectivity in a large number of participants with and without ASD from childhood to adulthood. Three main findings are reported. First, we identify a global connectivity pattern that represents a mean difference in connectivity pattern between ASD and TD groups. Second, age (from 7 to 40 years) did not contribute significantly to these findings in either group. Third, we find no specific increases in connectivity in ASD correlating with ASD symptomology.

Global Inter- Versus Intra-Network Connectivity

Results of the present study underscore the importance of evaluating *global* connectivity patterns in ASD, rather than individual networks. Weaker network segregation differentiated ASD and TD groups. Specifically, a combination of reduced intra-network and increased inter-network connectivity was evident in children through to adults with ASD, compared to controls. As shown in both Fig. 2a, b, decreased connectivity within the ventral attention, default mode and MOT was observed in the ASD group. Conversely, increased

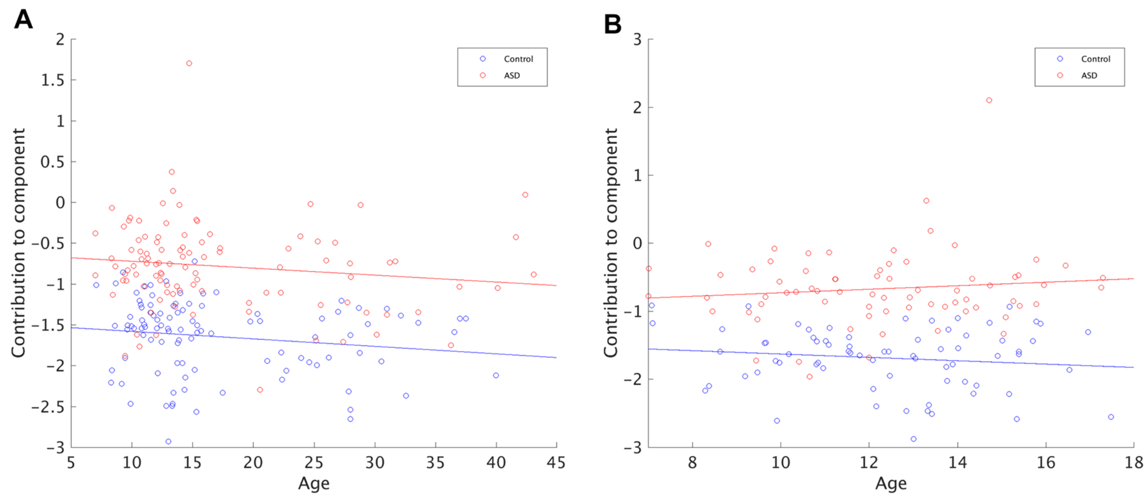

Fig. 3 PLS scores by age for **a** the 7–40 year-old group difference analysis and **b** the 7–18 year-old group difference analysis

connectivity was observed between several networks. Within these inter-network connections, the involvement of the motor and default mode networks was most evident. Many reports have noted atypical DMN intra-network connectivity in ASD (Assaf et al. 2010; Chien et al. 2015; Washington et al. 2014) as well in the sensorimotor regions (Nebel et al. 2014, 2016). Also, Chen et al. (2015) reported that in the top connectivity features for classification of ASD, 95% were internetwork. Atypicalities have also been reported in the MOT in ASD (Carper et al. 2015; Floris et al. 2016; Nebel et al. 2014, 2016) and are thought to relate to the prevalence of motor deficits in ASD—and can be one of the earliest signs in young children who go onto develop ASD. Fishman et al. 2014 studied the mentalizing network and also found a pattern of increased connectivity between functional networks. They referred to this effect as pathological cross-talk between networks, which is consistent with our findings of greater inter-network connectivity in ASD, but greater intra-network connectivity in controls.

Similar patterns of interactions have been reported in normative development. Specifically, functional brain development has been associated with a shift from local to distributed connectivity (Fair et al. 2009), where local segregation (weakening of local connections) and global network integration (strengthening of network connections within networks) has been used to predict individual brain maturation (Dosenbach et al. 2011). The weakening of inter-network connections was the most significant predictor of brain maturation. Poorer segregation between networks has been reported in children with other neurological disorders affecting cognition, such as epilepsy (Ibrahim et al. 2014). Thus, impaired network segregation may be a common characteristic of several neurodevelopmental disorders, including individuals with ASD.

Examining connectivity strengths organized by network across several networks provides insight into previous reports of over- and under-connectivity in ASD (Kana et al. 2011; Vissers et al. 2012). A *network-based view* of these connections is also critical to understanding brain function as it is unlikely to be localised or network-specific atypicalities that lead to connectivity disturbances. Our findings suggest that poor network segregation globally may contribute to the social-cognitive deficits, as measured by symptom severity, in ASD.

Network-Specific Decreases in Connectivity in ASD

While the global pattern of network interactions is essential when interpreting functional connectivity group differences, we also highlight important network-specific findings in those with ASD. Previous studies have shown that connectivity involving the DMN can differentiate ASD and control groups, as well as correlate with ASD symptomology (Assaf et al. 2010; Doyle-Thomas et al. 2015; Kennedy et al. 2006). Findings from the present study show that connections within the DMN are significant contributors to the differentiation of ASD and TD groups. These findings add to existing evidence that the DMN is crucial for normal brain function (Doyle-Thomas et al. 2015; Raichle, 2015), and that disruptions within this network may be associated with various neurodevelopmental disorders. Importantly, our study shows that although reduced DMN connectivity has been reported in ASD, it may be the connectivity patterns across multiple networks in combination with the DMN that characterizes ASD and explains the variance of symptom severity.

Network-Specific Increases in Connectivity in ASD

In the present work we did not identify significant associations between ASD symptomatology and within-network connectivity. Although bootstrapping showed significant increases in the salience network, the component itself was not significant.

The inter-network connectivity between the language and VIS contributed to the differentiation pattern of the children and adolescents with ASD from the TD group (see Fig. 2b). Impairments in language, communication and social interaction are key deficits that characterize ASD, and findings from previous studies have demonstrated abnormal neural function underlying language networks (Verly et al. 2014a, b). Given that atypical language function is prevalent in ASD, findings of abnormal connectivity between the language and VIS in ASD as part of global connectivity patterns may be particularly relevant in understanding the neurobiological underpinnings of this common symptom. Although our ASD group only included high-functioning children through adults, with verbal and reading skills, future work, including fMRI studies with language, reading and visual tasks, will help broaden our understanding of these results.

A limitation of this study included having only a 5 minute resting state, as recent reports suggest a longer recording period is valuable (e.g., (Birn et al. 2013)). However, 5 minutes lying still can seem like an eternity to young children and longer resting states would be very difficult for young and clinical populations; many would not be able to stay still for longer periods and their data would be lost to motion. A second limitation was the lack of significance in the analyses of the ADOS severity scores. As the pattern that emerged was consistent with a number of other significant studies in the field, we have included some discussion of this result, but recommend replication to verify these findings. It is noteworthy to also highlight the controversy of global signal regression in fMRI preprocessing (*for a review, see* (Murphy and Fox 2017)). There has been no consensus on one *correct* way to preprocess resting state fMRI data to most accurately model brain activity. Some have shown it may increase the degree of negative correlations, or reduce spatial accuracy of correlations, while others argue it can improve specificity of positive correlations and help remove non-neuronal signal components such as respiration (Murphy and Fox 2017).

In conclusion, in the present study we show that an overall pattern of stronger inter-network and weaker intra-network in resting state fMRI correlations differentiated the ASD from the TD group. This study significantly extends prior work proposing that neural network-level associations with ASD are not limited to individual, distinct networks, such as the default mode and MOT, but can be described by a more global network disruption. The DMN was the strongest contributor to both group differentiation analyses. In addition, consistent with previous studies, the MOT had robust contributions to the differentiation, with both inter- and intra-network abnormalities. These patterns of inter- and intra-network connections are similar to those found in developmental literature, which may reflect immature, delayed, or abnormal functional network development in those with ASD. The PLS approach used in this study allowed for the observation of global network patterns in ASD and the significant findings are relevant to understanding ASD as a disruption of typical network topology and segregation.

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