Defining subtypes of autism spectrum disorder using static and dynamic functional connectivity

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ABSTRACT

Autism spectrum disorder (ASD) is a highly heterogeneous neurodevelopmental disorder that is characterized by impairments in social communication and restricted and repetitive behaviours. Neuroimaging studies of individuals with ASD have shown complex patterns of functional connectivity (FC), with no clear consensus on defining brain-behaviour attributes of different subtypes of the disorder. In these studies, “static FC”, a metric that considers correlations across the entire time series for a given region, was used. Emerging evidence shows that FC changes over time; this “dynamic FC” can allow for unique detection of the temporal variability of functional connections over time. Here, we used k-means clustering, an unsupervised machine learning algorithm, to characterize two subtypes of ASD based on distinct patterns of static and dynamic FC. A multivariate statistical approach was then implemented to determine optimal relationships between FC patterns and group membership or behaviour. The main objective was to characterize differences in static and dynamic FC between subtypes of ASD defined in a fully data-driven manner, and between subtypes and typically developing individuals. Subtype 2 was defined by increased FC within resting-state networks and decreased FC across networks for static FC, global increases in temporal stability of dynamic FC, and robust relationships between FC and several behaviours, relative to Subtype 1. Further, both ASD subtypes exhibited different patterns of static and dynamic FC compared to controls, particularly within default mode, sensorimotor, and occipital networks. Static and dynamic FC metrics provided both overlapping and unique information about the nature of FC differences between subtypes, and between both subtypes and controls. Our results demonstrate the value of considering FC-based subtypes of ASD to elucidate different relationships between brain and behaviour among individuals with this disorder, and have important clinical implications for catering treatments and behavioural interventions to specific subtypes.

Keywords: autism spectrum disorder, static functional connectivity, dynamic functional connectivity, unsupervised machine learning, multivariate analysis

Abbreviations:

ABIDE, Autism Brain Image Data Exchange; ADI-R, Autism Diagnostic Interview Revised; ADOS, Autism Diagnostic Observation Scale; ASD, autism spectrum disorder; BSR, bootstrap ratio; CER, cerebellar network; COMM, communication; CON, cingulo-opercular network; Cov. = covariance; DMN, default mode network; FC, functional connectivity; FD, framewise displacement; FPN, frontoparietal network; LV, latent variable; OCC, occipital network; PCP, Preprocessed Connectomes Project; PLS, partial least squares; PLS-B, behaviour partial least squares; PLS-MC, mean-centering partial least squares; ROI, region of interest; RRBs, restricted and repetitive behaviours; rs, resting-state; RSN, resting-state network; SA, social affect; SMN, sensorimotor network; SRS, Social Responsiveness Scale; SVD, singular value decomposition; TD, typically developing

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social cognition as well as restricted and repetitive behaviours (RRBs; American Psychiatric Association, 2013). ASD is a highly heterogeneous disorder, with a broad range of the types and severities of behaviours that can be displayed. For instance, verbal and nonverbal IQ is highly variable in ASD (e.g. Munson et al., 2008), and RRBs can range from low-level motor stereotypies to higher-order behaviours such as insistence on sameness (American Psychiatric Association, 2013). It has been proposed that these complex behavioural features may arise from changes in brain network dynamics. Several theories focus on atypical patterns of "static" functional connectivity (FC), which is measured across entire fMRI scan lengths and therefore provides a temporally global perspective of communication between brain regions. Such theories include reduced communication between frontal and posterior brain regions (Just et al., 2012), increased local FC along with reduced long-range FC (Belmonte et al., 2004; Courchesne & Pierce, 2005), and an abnormal developmental trajectory of FC in relation to typically developing (TD) individuals (Nomi & Uddin, 2015; Uddin et al., 2013b). However, complex patterns of both increased and decreased FC have been found in neuroimaging studies of ASD, and results are inconsistent across studies (see Hull et al., 2016; Picci et al., 2016; and Uddin et al., 2013b for reviews). Further, there has yet to be a framework describing atypical FC in ASD that is supported consistently across studies.

To date, the majority of resting state fMRI studies of FC in ASD have examined static FC. However, functional connections dynamically reconfigure over short time periods (Allen et al., 2014; Hansen et al., 2015; Hutchison et al., 2013a, 2013b). "Dynamic" FC measures how FC strength changes over the length of a scan, therefore providing information about temporal stability or variability of FC and changes in brain states. It is particularly valuable to study dynamic FC in ASD, as it has been suggested that the disorder is characterized by a decreased signal to noise ratio in the brain; thus, noisier, more variable signaling can be expected (Rubenstein & Merzenich, 2003) and may mediate the atypical patterns of static FC (Falahpour et al., 2016). While little research has been conducted on the dynamic nature of FC in individuals with ASD, recent studies have reported increased variability of FC, particularly for connections involving the default mode network (Chen et al., 2017; Falahpour et al., 2016; Zhang et al.,

2016). While dynamic FC is related to static FC in that weaker static connections exhibit greater temporal variability (e.g. Betzel et al., 2016; Falahpour et al., 2016; Li et al., 2015; Thompson & Fransson, 2015), it is possible that measures of dynamic FC can provide unique information about the nature of FC in ASD that cannot be provided by measures of static FC alone.

Further, studies have reported mixed results regarding relationships between FC abnormalities and behavioural profiles in individuals with ASD (e.g. Hahamy et al., 2015; Keown et al., 2013; Lee et al., 2016; Monk et al., 2009; Uddin et al., 2013b). Defining subtypes of ASD based on FC metrics has the potential to resolve some of the current discrepancies in the literature regarding the nature of FC abnormalities in individuals with this disorder, as well as to shed light on the complex relationships between FC and behaviour, which may differ between meaningful subtypes. Previously, ASD subtypes have been defined based on clusters of social communication behaviours and RRBs (Georgiades et al., 2012), structural MRI (Hrdlicka et al., 2005), and static FC (Chen et al., 2015), but have not yet been defined using dynamic FC. Further, previous characterization of ASD subtypes based on static FC (Chen et al., 2015) involved two subtypes that differed in terms of ASD symptom severity. It is necessary to consider brain-based methods for defining subtypes and their relationships with behavioural profiles in individuals with ASD, as it could be the case that certain behaviours result from complex interplay between local and distributed processing the brain. In addition, FC-based subtypes that do not differ in symptom severity would reveal information about meaningful differences in FC between individuals with ASD that are independent of simple behavioural measures of overall severity.

In the present study, we used a data-driven approach to characterize subtypes of ASD based on distinct clusters of static and dynamic FC, and to relate FC patterns to specific behavioural profiles in these subtypes. We first used unsupervised machine learning to define subtypes of ASD using static and dynamic FC, and implemented a multivariate statistical analysis that, when applied to neuroimaging data, reveals the optimal relationship between measures of brain activity and experimental design or group membership. This approach allowed us to determine which static and dynamic connections were reliably different between ASD subtypes, and between ASD subtypes and TD participants. We also used this multivariate approach to characterize relationships between particular patterns of FC and a set of behaviours. It was hypothesized that defining subtypes of ASD using data-driven metrics would reveal

unique information about brain-behaviour interactions in this highly heterogeneous clinical population.

2. Materials and methods

2.1. Participants

Resting-state fMRI (rs-fMRI) data from 163 males with ASD and 110 TD males were acquired from the Preprocessed Connectomes Project (PCP; Craddock et al., 2015; http://www.preprocessed-connectomes-project.org/abide). The data had been obtained from the Autism Brain Imaging Data Exchange (ABIDE; Di Martino et al., 2014; http://www.fcon_1000.projects.nitrc.org/indi/abide) and preprocessed using the Connectome Computation System pipeline (Xu et al., 2015). Participants were excluded if their full scale IQ was less than or equal to 75 or if their mean framewise displacement (FD) during the rs-fMRI scan was greater than 0.20mm. Children were matched for age, IQ, and mean framewise displacement (Table 1).

Table 1: Participant characteristics

Variable (Mean ± SD)	ASD	TD	Significance
N	163	110	
Age	16.60 ± 6.69	16.25 ± 5.94	t (271) = 0.4451, p = 0.6566
IQ	106.86 ± 16.62	107.27 ± 11.61	t (271) = -0.2257, p = 0.82
Mean FD	0.085 ± 0.047	0.077 ± 0.034	t (271) = 1.6015 p = 0.11

ASD diagnoses were confirmed using the Autism Diagnostic Observation Scale (ADOS; Lord et al., 2000) and/or the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994). Scores from these scales are presented in Supplementary Table 1.

2.2. fMRI Preprocessing

Data from five sites (New York University Lagone Medical Center, University of Utah School of Medicine, San Diego State University, Trinity Centre for Health Sciences, and University of Michigan (Sample 1)) using a TR of 2000ms and that did not use data scrubbing to eliminate high-motion data points were included. These criteria were important in order to maintain the temporal structure of the data for dynamic FC. Written informed consent or assent was obtained for all participants in accordance with respective institutional review boards. Additional scanning parameters can be found on the ABIDE website (http://www.fcon_1000.projects.nitrc.org/indi/abide). Preprocessing included dropping the first 4 volumes, slice timing correction, motion realignment, intensity normalization, 24-parameter motion regression, and regressing out mean white matter and CSF signals as well as linear and quadratic trends. Data were bandpass filtered from 0.01 to 0.1 Hz. The global signal was regressed from the data, as this step has been shown to help mitigate differences across multiple sites (Power et al., 2014). A set of functional-to-anatomical and anatomical-to-template transforms was used to spatially normalize images to the MNI152 template. The time series of 160 4.5mm spherical regions of interest (ROIs) from the Dosenbach atlas (Dosenbach et al., 2010) were obtained. Regions in this atlas were selected from meta-analyses of task-related fMRI studies and categorized into six different resting-state networks (RSNs): the default mode network (DMN), frontoparietal network (FPN), cingulo-opercular network (CON), occipital network (OCC), sensorimotor network (SMN), and cerebellar network (CER). Additional details of the fMRI preprocessing steps can be found on the PCP website (http://www.preprocessed-connectomes-project.org/abide).

2.3. Functional connectivity

Static FC was defined by Fisher z-transformed Pearson correlations for each ROI pair across all time points for each participant. For dynamic FC, the procedure for calculating static FC was applied using a sliding window approach, using a window size of 15 TRs (30 seconds). This window size was chosen based on previous literature demonstrating that window sizes between 30 and 64s were associated with lower variability of prediction errors, lower permutation p-values and higher stability of bootstrap ratios when using multivariate approaches to examine the association between FC variability and chronological age (Qin et al., 2015). This sliding window size has also been used in previous studies of dynamic FC (Allen et al., 2014;

Hutchison et al., 2013b; Shen et al., 2015). The sliding windows were moved in increments of one time point across the time series. The average of the normalized autocorrelation sequence of the time series of each connection across time lags greater than or equal to zero was then calculated (Shen et al., 2015). This metric is referred to as "temporal stability", as a larger autocorrelation coefficient indicates lower temporal variability, that is, higher temporal stability of dynamic FC. The effects of age, head motion, and acquisition site (represented as a Helmert basis) were regressed out of the FC matrices.

2.4. K-means Clustering

K-means clustering was used to define two subtypes of ASD. The lower triangle of each participant's static and dynamic FC matrices was used, such that the matrix for k-means was in the form subjects x FC, where static and dynamic FC were concatenated. The k-means algorithm begins with an initialization of *k* centroids. Then, in the *assignment* step, each participant is assigned to the closest centroid using the cosine distance, defined as one minus the cosine of the included angle between each subjects' FC values and each cluster's centroids, which are treated as vectors. Next, in the *centroid update* step, new centroids are defined as the mean of the data points that are currently assigned to that centroid. These two steps are repeated iteratively until convergence, when cluster assignments no longer change.

The "elbow point" criterion was used to determine the optimal number of clusters for the ASD dataset. To determine the elbow point, the average cosine distance between a cluster's centroids and the FC values of participants assigned to that particular cluster is calculated for each cluster, then averaged across clusters to obtain a single distance metric for each value of k. These distances are then plotted as a function of k, and the "elbow" is defined as the value of k where the change in the rate of decrease in distance is sharpest. Values from k = 2 to k = 8 were tested (but also included k = 1 in the elbow point plot as a reference point), so that the average number of participants in each cluster would be no less than 20.

2.5. Partial Least Squares

Partial least squares (PLS) is a multivariate statistical technique that is used to optimally relate brain activity to experimental design or group membership in the form of latent variables (LVs; McIntosh et al., 1996; McIntosh & Lobaugh, 2004; Krishnan et al., 2011). In *mean-*

centering PLS (PLS-MC), patterns relating a matrix of brain variables (in the form subjects x brain variables) and group membership are calculated. For this study, the brain variables were either static FC, or the temporal stability of dynamic FC for each connection in the lower triangle of each subject's FC matrix (12720 connections). PLS-MC was used to examine differences in static and dynamic FC between the two ASD subtypes defined by k-means and TD individuals.

Using singular value decomposition (SVD), orthogonal patterns that express the maximal covariance between the brain variables and group membership are computed. Each pattern consists of saliences (weights) and a singular value. The brain saliences indicate which brain variables (in this case, functional connections) best characterize the relationship between the brain variables and group differences. Design saliences indicate the group differences profiles that best characterize this relationship. Singular values indicate the proportion of covariance between the brain and design matrices that each pattern accounts for. Brain scores, which represent each subject's contribution to each brain salience, are calculated by multiplying the original matrix of brain variables by the brain salience.

In *behaviour PLS* (PLS-B), a matrix of behaviour variables is also included in the analysis to determine design-dependent (in this case, group-dependent) relationships between the brain variables and behaviour. For this study, behavioural PLS was used to examine associations between static and dynamic FC and a set of behavioural variables including IQ, ADOS scores (communication, social affect, and RRBs), and scores on the Social Responsiveness Scale (SRS) between the two ASD subtypes.

The statistical significance of each pattern was determined using permutation testing. For this procedure, the rows (participants) of the matrix of brain variables are reshuffled, and new singular values are obtained using SVD. In this study, this procedure was repeated 1000 times to create a distribution of singular values. The p-value associated with the original singular value is defined as the proportion of singular values from the sampling distribution that are greater than the original singular value, thus representing the probability of obtaining a singular value larger than the original value under the null hypothesis that there is no association between the brain variables and group membership.

In addition to determining the statistical significance of each pattern, the reliability of the brain saliences can also be determined by utilizing a bootstrapping procedure. Bootstrap samples are generated by randomly sampling subjects with replacement, while ensuring that group

membership is maintained. In this study, 500 bootstrap samples were generated. Creating bootstrap samples allows one to determine which brain variables are stable, regardless of which participants are included in the analysis. The bootstrap ratio (BSR), defined as the ratio of the brain salience to the standard error of the salience (as estimated by the bootstrap procedure), is a measure of this stability. Reliable connections were defined as those that surpassed a BSR threshold of ± 2.0, which corresponds roughly to a 95% confidence interval.

To assess the extent to which ROIs that exhibited reliable group differences for static FC also exhibited group differences for dynamic FC, regardless of the direction of the difference, the absolute values of the saliences for connections with a BSR greater than 2 or less than -2 for both the static and dynamic FC analyses were correlated. The significance of each correlation was assessed by creating a distribution of r-values after permuting the order of the connections in the dynamic FC salience vector, then calculating the p-value as the proportion of r-values from the distribution that were greater than or equal to the original correlation.

In addition to assessing the contribution of each individual connection to the group differences, we were interested in determining the extent to which network-level FC, both within and between RSNs, contributed to the group differences. This was of particular interest due to hypotheses that ASD may be characterized by atypical FC within and between networks (e.g. Hull et al., 2016; Rudie & Dapretto, 2013b). To assess the relative contributions of each RSN to the spatial patterns, the BSR-thresholded spatial maps (i.e. adjacency matrices in the form connections x connections) were separated into positive BSRs and negative BSRs. These maps were thresholded such that connections with a BSR less than 2 but greater than -2 were set to 0. Positive BSRs greater than 2 were set to 1, and negative BSRs less than -2 were set to -1. All thresholded BSRs within each pair of networks were then averaged to obtain a 6x6 matrix showing the average contribution of each network pair to the spatial pattern, separately for positive and negative BSRs. To assess the significance of these contributions, the order of connections in the BSR thresholded matrices was permuted while keeping the RSN labels the same, and then the above procedure was repeated to calculate the RSN contributions. This process was repeated 1000 times to obtain a distribution of average contribution values for each RSN pair. Then, the significance of the original contribution is defined as the proportion of contribution values from the sampling distribution that are greater than or equal to the original

value. This procedure was repeated for static and dynamic connections that uniquely contributed to the LVs, and connections that contributed to the LVs for both static and dynamic FC.

3. Results

3.1. K-means clustering

The optimal number of clusters for the ASD dataset, as determined by the elbow point criterion, was 2 (Fig. 1).

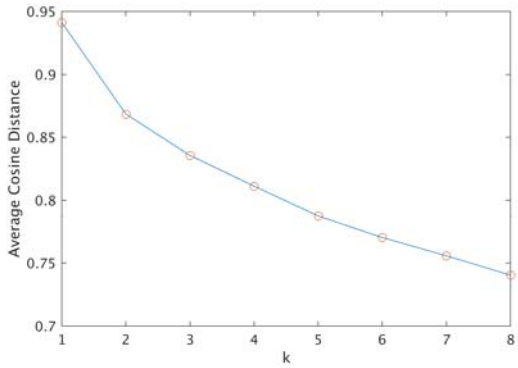

Fig. 1: Elbow point plot, indicating that the optimal number of clusters is 2.

The k-means algorithm converged after 9 iterations on a solution that included 92 participants in Subtype 1 and 71 participants in Subtype 2. The centroids of FC values for each subtype are shown in Fig. 2. Qualitatively, it can be seen that Subtype 1 was defined by stronger static FC between networks, particularly between the DMN and other networks, and weaker static FC within networks relative to Subtype 2. Further, Subtype 1 showed overall weaker stability of dynamic FC compared to Subtype 2, which was particularly evident within the occipital network. Importantly, subtypes did not differ in ASD symptom severity, IQ, eye status, medication use, presence of comorbidities, or the parameters (age, head motion, and scan site) that were regressed out of the FC matrices (see Supplementary Table 3). Differences in FC between subtypes were then analyzed statistically using PLS-MC.

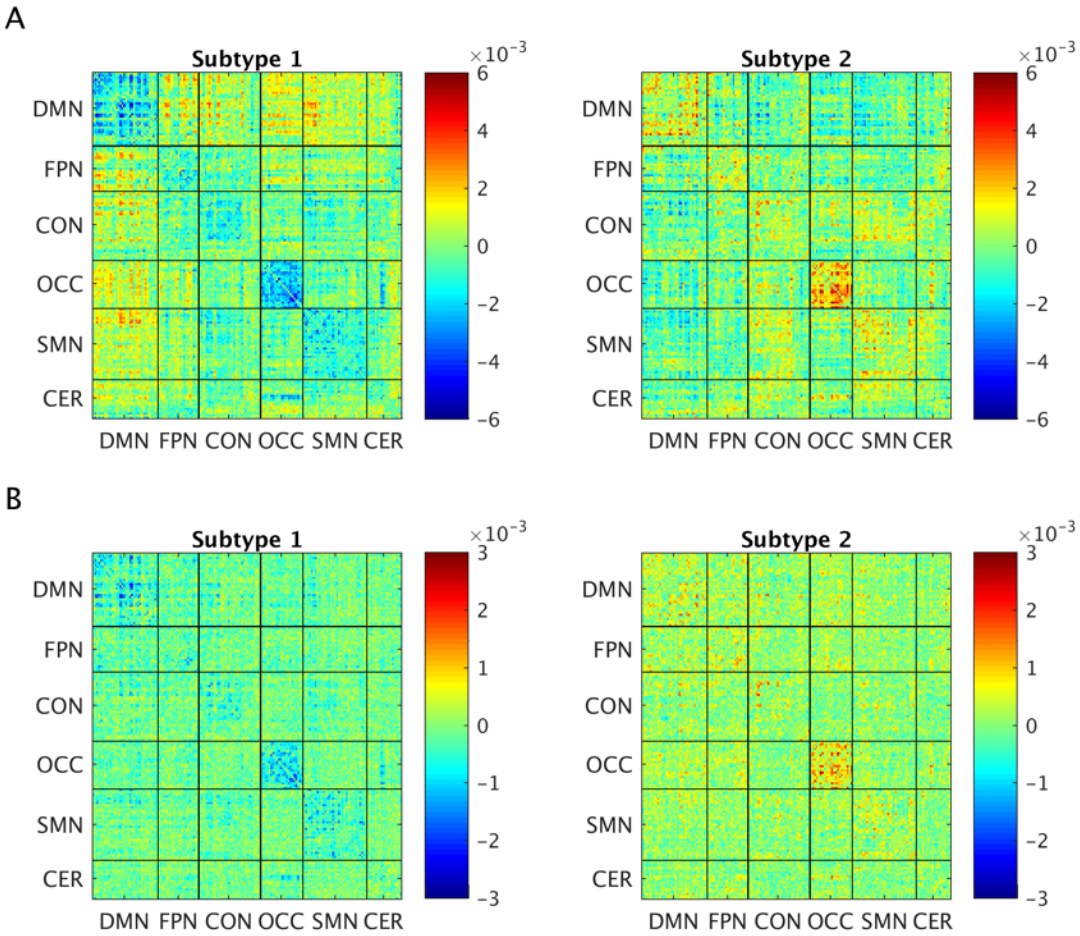

Fig. 2. Centroids for ASD subtypes for A) static FC and B) temporal stability of dynamic FC, grouped into RSNs. DMN = default mode network, FPN = frontoparietal network, CON = cingulo-opercular network, OCC = occipital network, SMN = sensorimotor network, CER = cerebellar network.

3.2. Multivariate Analyses of Static and Dynamic Functional Connectivity

For both static and dynamic FC, PLS-MC revealed two significant LVs. The first LV was a contrast between the two ASD subtypes, and the second was a contrast between both subtypes and the TD group. Thus, the first LV is essentially a statistical confirmation of the FC centroids for each subtype, which would be expected to be significant since the two subtypes were defined to express different patterns of FC.

LV1 for static FC revealed reliable differences between subtypes in within- and between-network FC ($p < 0.0001$, 70.11% covariance explained). Positive BSRs showed that individuals in Subtype 1 exhibited reliably greater FC between the DMN and other networks, and negative BSRs indicated that Subtype 2 exhibited greater FC within networks (Fig. 3).

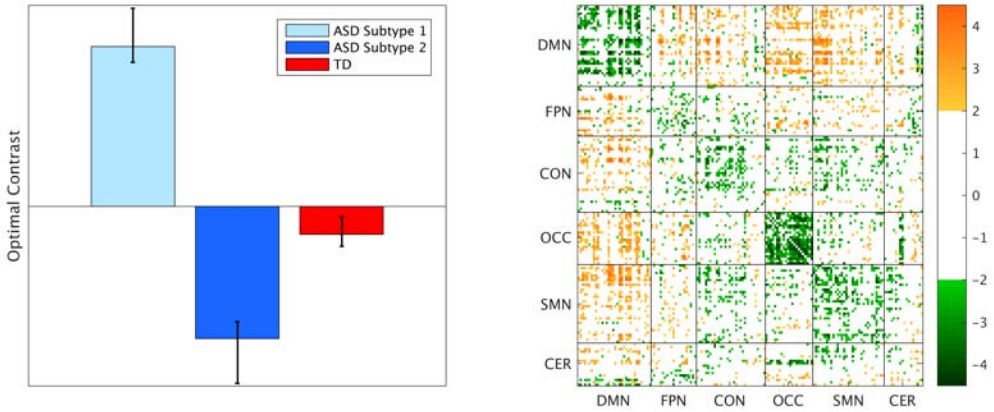

Fig. 3. LV1 for static FC and the associated BSRs for each connection, at a threshold of ± 2.

We then evaluated the group-averaged expression of each contrast. Examining the positive expression of the contrast (Fig. 4A), we found that connections were positive for Subtype 1 and negative for Subtype 2. In other words, individuals in Subtype 2 tended to exhibit greater anti-correlations between resting state networks, especially between-network connections involving the DMN, compared to Subtype 1. The negative expression of the contrast showed that Subtype 1 was characterized by negative connections on average within networks, whereas these connections were positive in Subtype 2 (Fig. 4B).

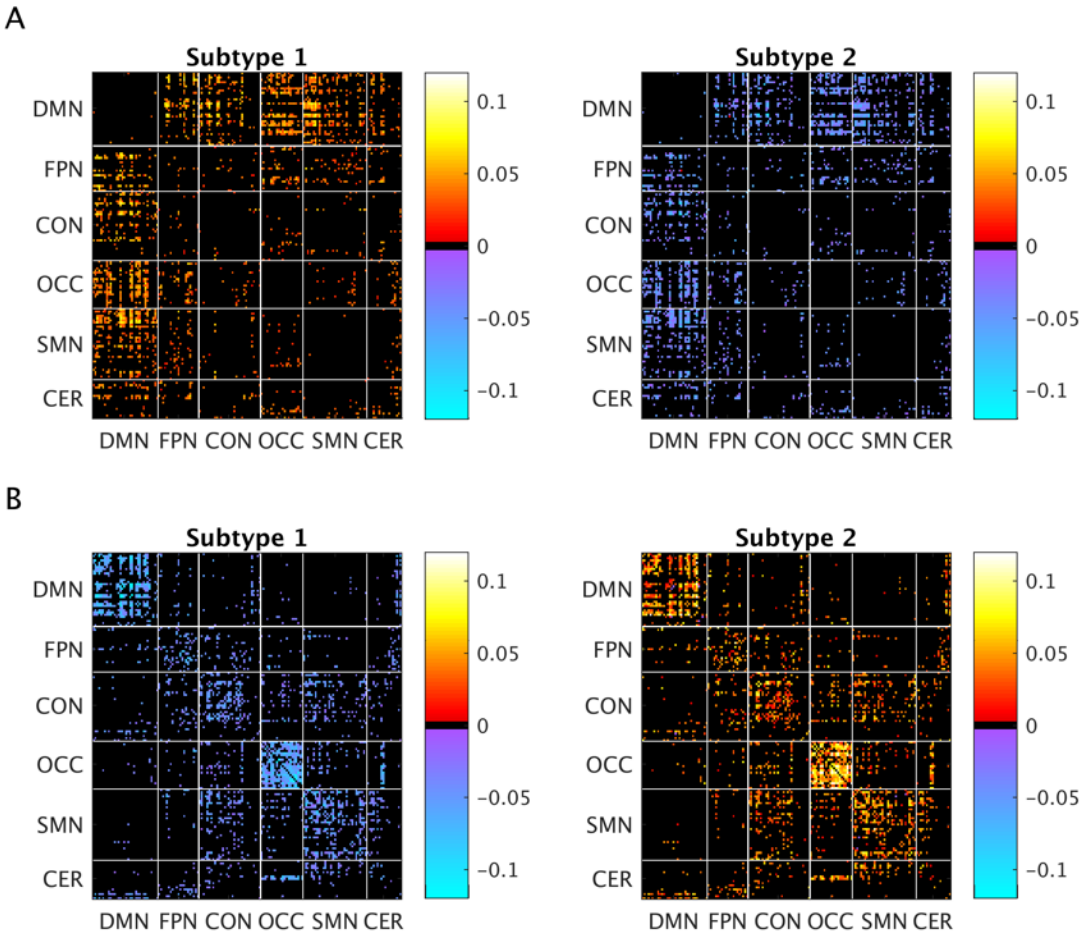

Fig. 4. Static FC contrast expressions for LV1, showing average Fisher z-transformed Pearson correlation coefficients for each group. A) Positive expression of the contrast (BSRs > 2). B) Negative expression of the contrast (BSRs < -2).

LV1 for dynamic FC ($p < 0.0001$, 65.53% covariance explained) revealed that Subtype 2 was characterized by overall greater temporal stability, both within and between RSNs (Fig. 5). Further, the absolute values of the static and dynamic FC brain saliences were highly correlated, $r = 0.7326$ ($p < 0.0001$), indicating that regions that contributed strongly to the static FC LV tended to also contribute strongly to the dynamic FC LV (Fig. 6A). Next, we determined which functional connections contributed reliably to the LV for both static and dynamic FC, and those that were unique to static FC or dynamic FC (Fig. 6B). The maximum overlap between static and dynamic FC occurred within the occipital network.

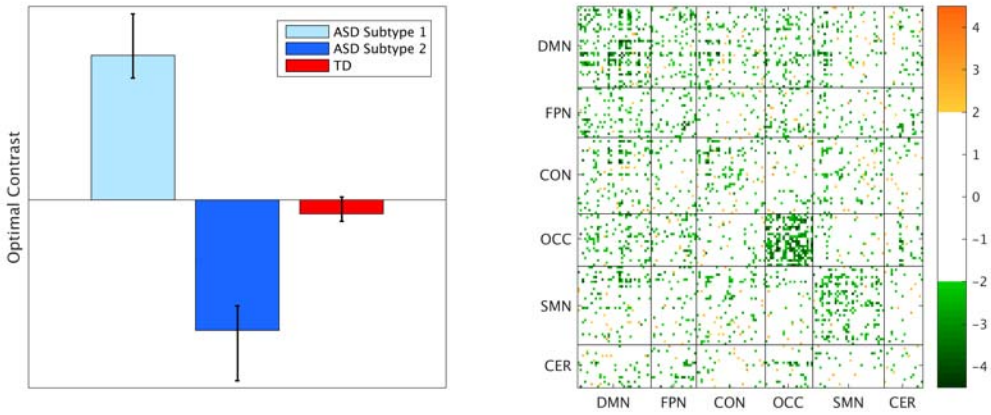

Fig. 5. LV1 for dynamic FC and the associated BSRs for each connection, at a threshold of ±2.

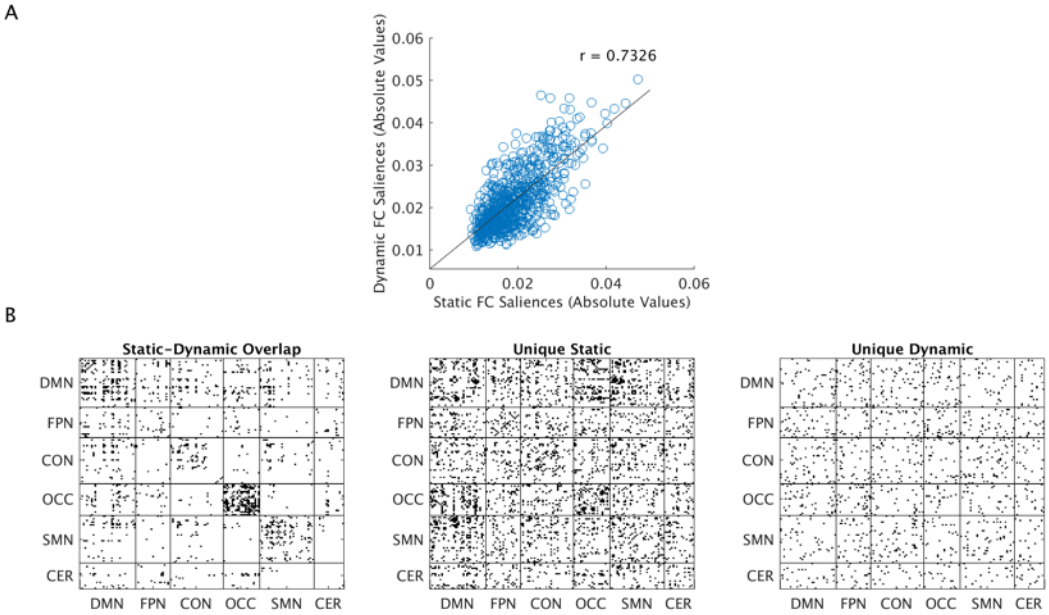

Fig. 6. A) Relationship between static and dynamic FC brain saliences for LV1. B) Overlapping and unique static and dynamic connections.

The second LV for static FC revealed a contrast between both ASD subtypes and the TD group ($p < 0.0001$, 29.89% covariance explained; Fig. 7). The positive expression of the contrast (Fig. 8A) showed that these connections were, on average, positive in the ASD subtypes and

negative in controls, whereas the opposite was true for the negative expression of the contrast (Fig. 8B).

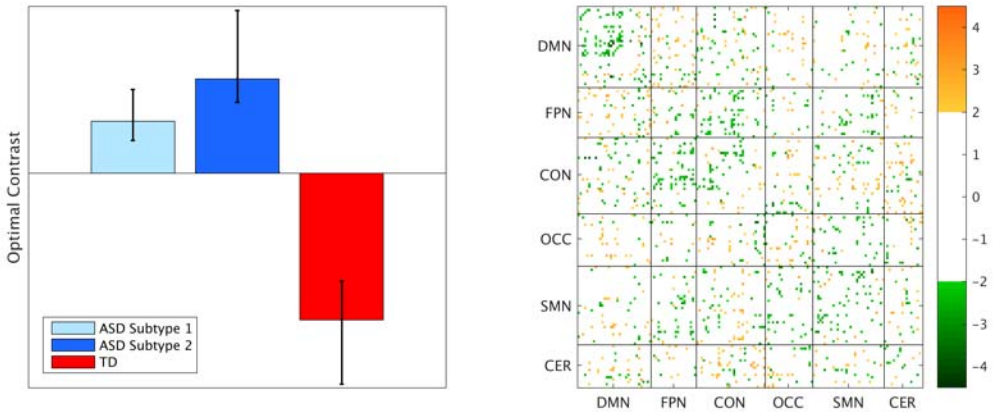

Fig. 7. LV2 for static FC and the associated BSRs for each connection, at a threshold of ±2.

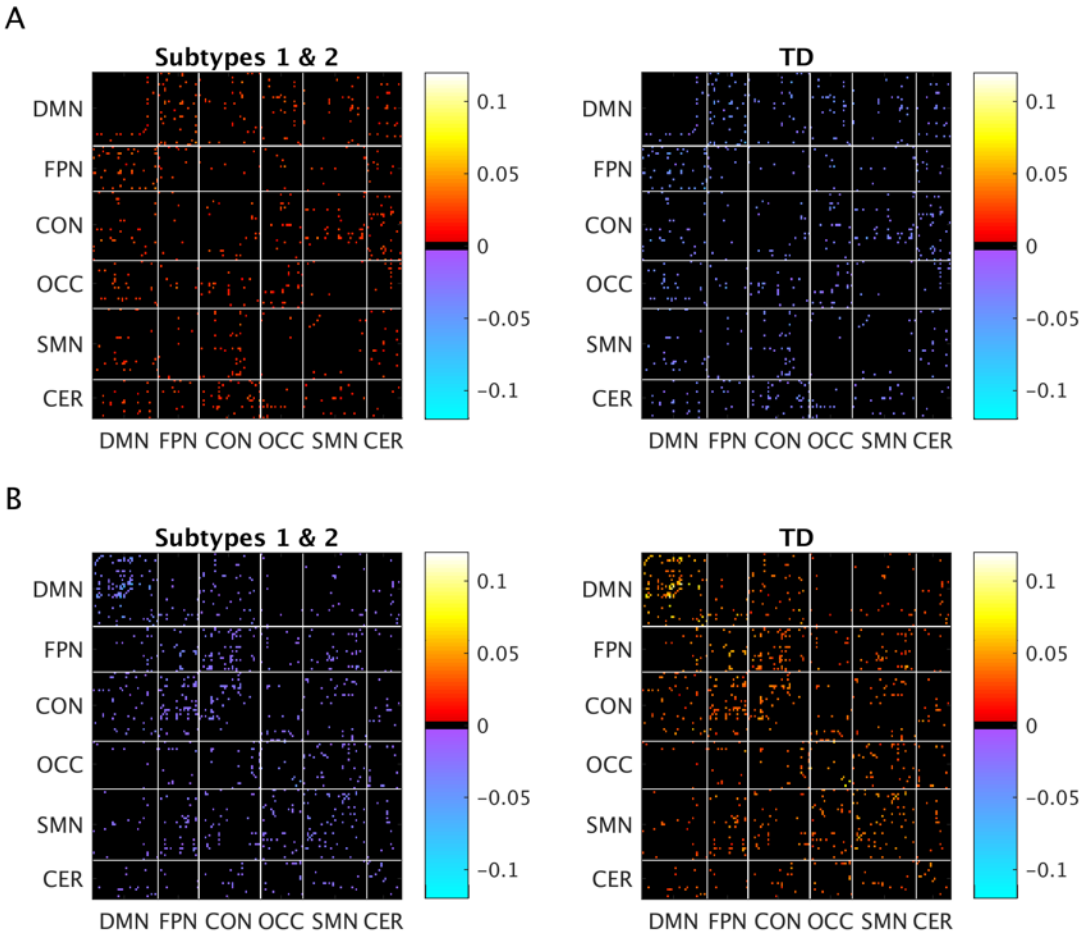

Fig. 8. Static FC contrast expressions for LV2. A) Positive expression of the contrast (BSRs > 2). B) Negative expression of the contrast (BSRs < -2).

The second LV for dynamic FC (p = 0.006, 34.47% covariance explained) showed that both ASD subtypes exhibited patterns of both increases and decreases in temporal stability compared to the TD group (Fig. 9). The correlation between the absolute static and dynamic FC saliences was r = 0.6162 ($p < 0.0001$), indicating that the contributions of static and dynamic connections were qualitatively less similar for LV2 compared to LV1 (Fig. 10).

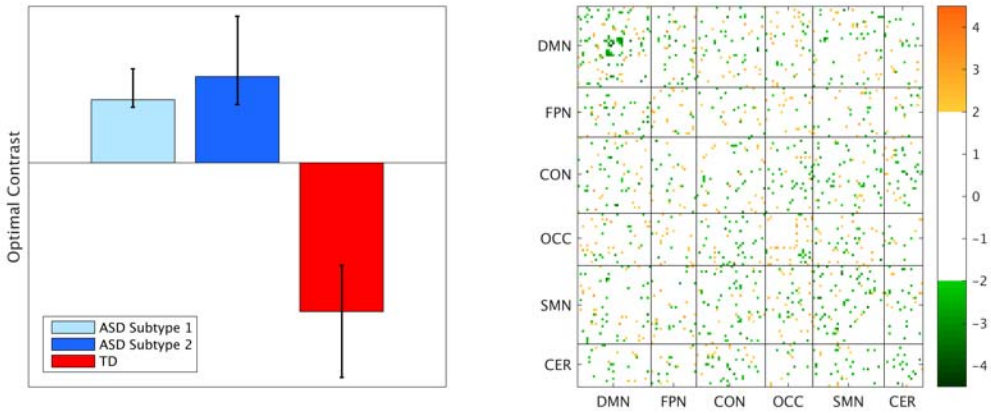

Fig. 9. LV2 for dynamic FC and the associated BSRs for each connection, at a threshold of ±2.

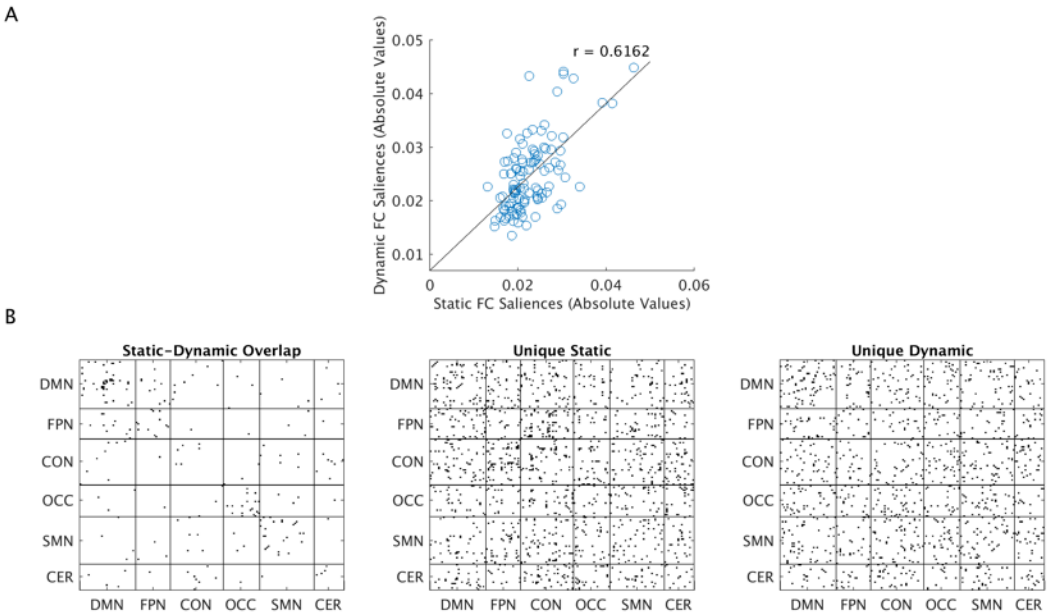

Fig. 10. A) Relationship between static and dynamic FC brain saliences for LV2. B) Overlapping and unique static and dynamic connections.

We used PLS-B to assess relationships between FC and a set of behavioural measures in the two ASD subtypes, including IQ, ADOS scores (communication (COMM), social affect (SA), and restricted and repetitive behaviours (RRB)), and scores on the Social Responsiveness scale (SRS). For both static and dynamic FC, there was one significant LV. The static FC LV ($p = 0.015$, 25.89% covariance explained) revealed reliable relationships between FC and all

behavioural measures in Subtype 2. These relationships were unreliable in Subtype 1, except for ADOS SA scores (Fig. 11). As shown by the contrast expressions, ADOS and SRS scores exhibited positive and negative correlations with certain sets of connections, whereas the opposite pattern was observed for IQ scores (Fig. 12).

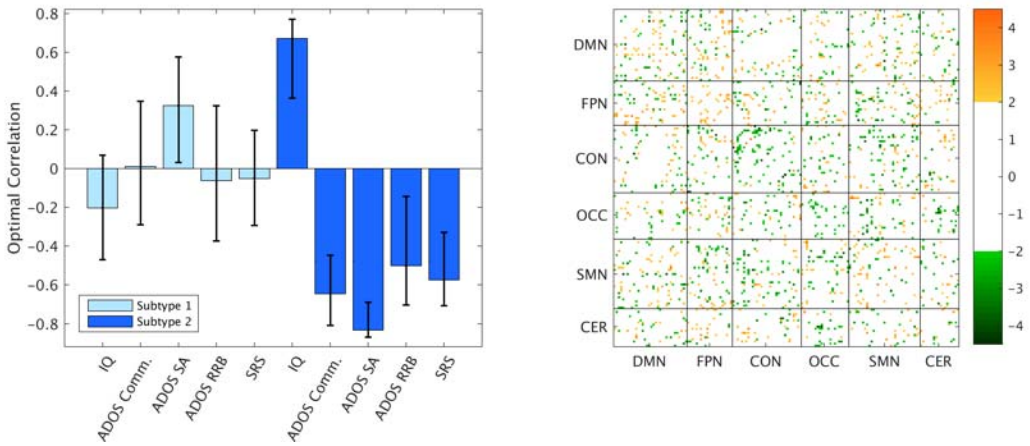

Fig. 11. The PLS-B LV for static FC and the associated BSRs for each connection, at a threshold of ± 2.

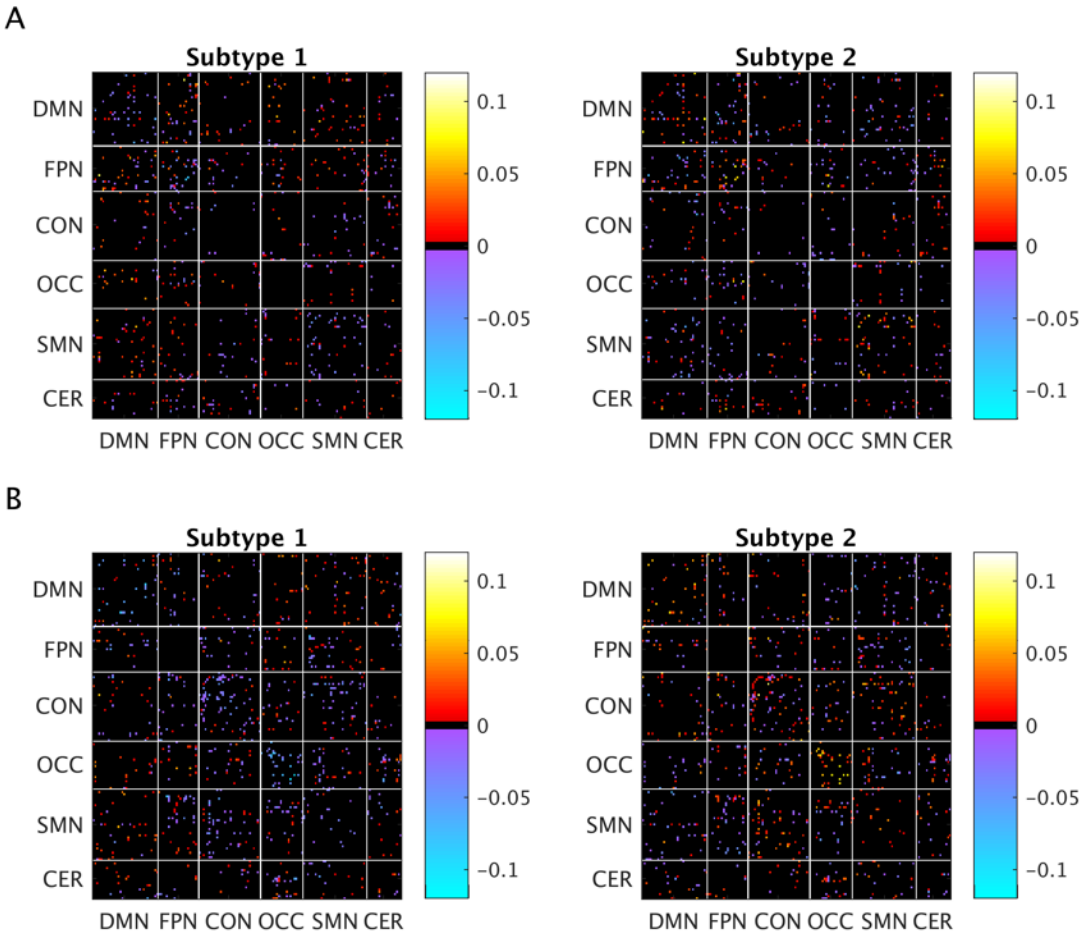

Fig. 12. Static FC contrast expressions. A) Positive expression of the contrast (BSRs > 2). B) Negative expression of the contrast (BSRs < -2).

A similar result was observed for PLS-B for dynamic FC (p = 0.041, 25.51% covariance explained), whereby FC-behaviour correlations were reliable in Subtype 2. For Subtype 1, only ADOS RRB scores showed a reliable relationship with a subset of connections (Fig. 13). The correlation between the absolute static FC and dynamic FC saliences was r = 0.3522 (*P* < 0.0001; Fig. 14).

Importantly, no reliable relationships between FC and behaviour were present when all ASD participants were grouped together, for both static FC (p = 0.1818) and dynamic FC (p = 0.4416).

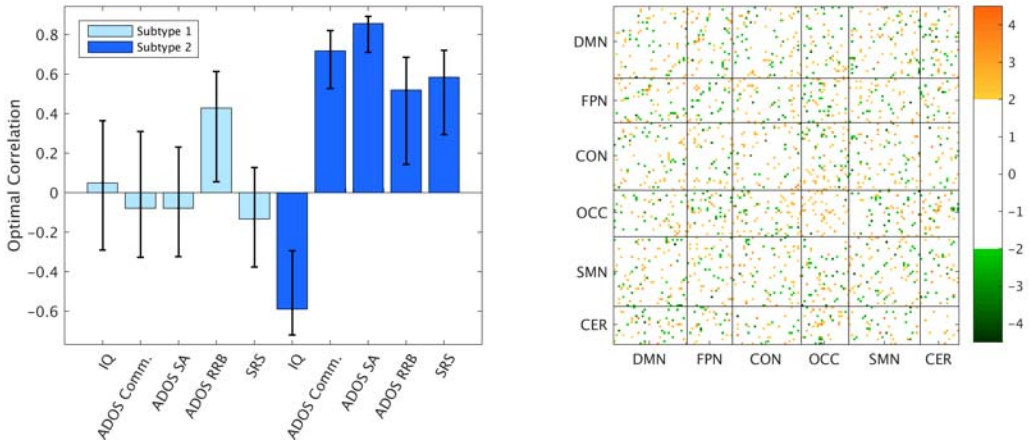

Fig. 13. The PLS-B LV for dynamic FC and the associated BSRs for each connection, at a threshold of ±2.

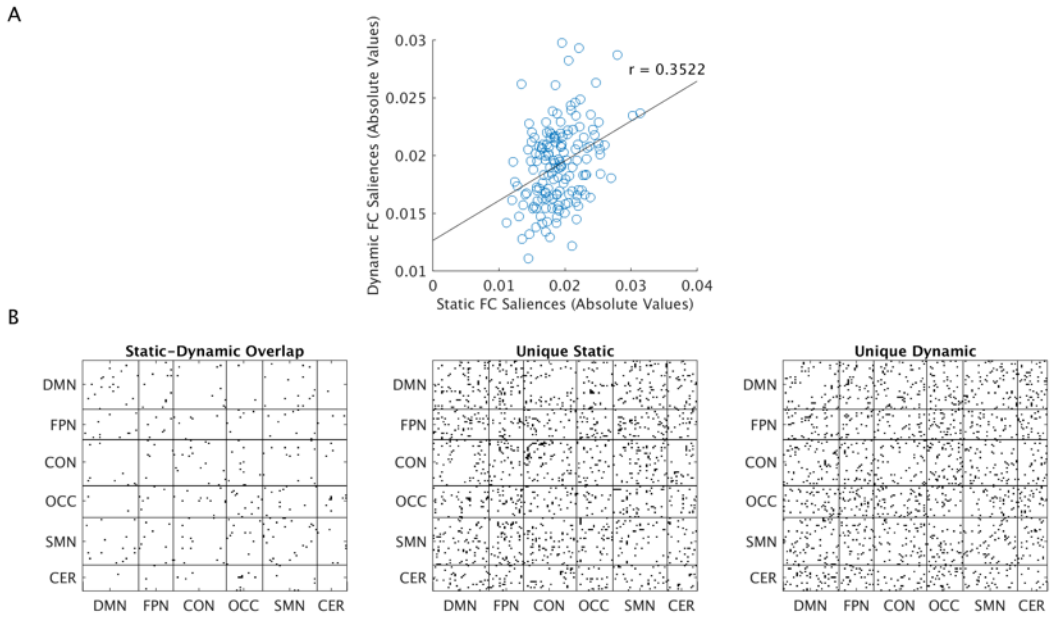

Fig. 14. A) Relationship between static and dynamic FC brain saliences for PLS-B. B) Overlapping and unique static and dynamic connections.

3.3. Relative Contributions of RSNs to Spatial Patterns

Fig. 15 summarizes which RSN pairs significantly contributed to each LV. Overall, the OCC made the greatest contribution to the LVs (15/24 possible significant contributions), followed by the DMN (13), FPN (12), SMN (10), CON (8), and finally CER (3). Fig. 16 shows the RSN pairs that contributed to each LV for unique and overlapping static and dynamic connections. MC-LV1 for static FC reveals that positive BSRs were only stable between certain networks, while negative BSRs were stable within all RSNs except the cerebellum, with one reliable set of between-network connections (CON-SMN). MC-LV1 for dynamic FC showed that only negative BSRs exhibited reliable network-level connectivity, for select within- and between-network connections.

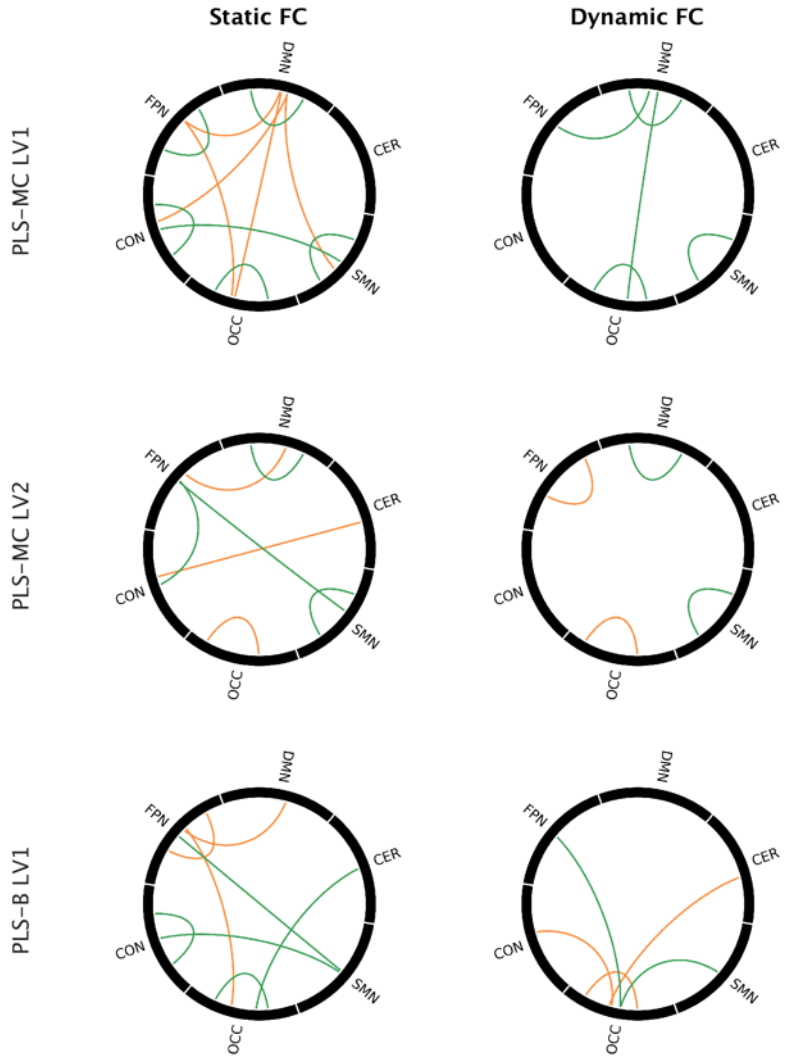

Fig. 15. Significant contributions of RSN pairs to each LV for positive and negative BSRs. Orange = positive BSRs, green = negative BSRs.

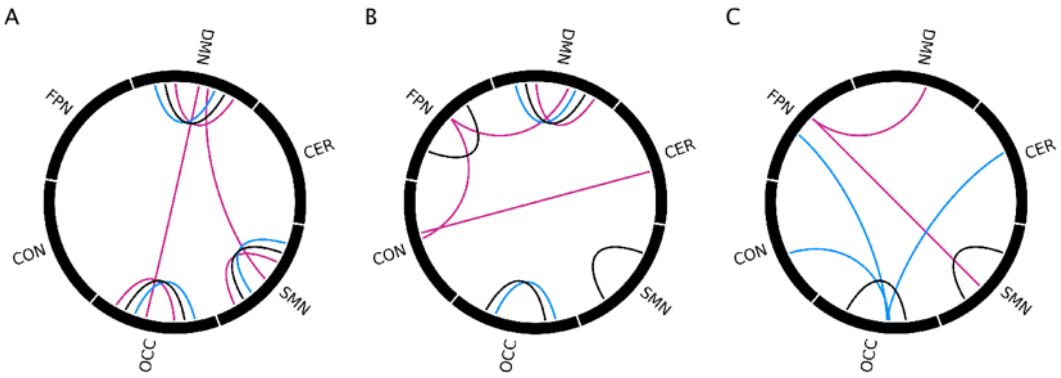

Fig. 16. Significant contributions of RSN pairs to each LV for unique and overlapping static and dynamic connections. A) MC-LV1, B) MC-LV2, C) B-LV1.
Black = overlap, pink = unique static, blue = unique dynamic.

4. Discussion

4.1. Overview

This study demonstrates that meaningful brain-based subtypes of ASD can be defined based on static and dynamic FC. Using a data-driven approach, we characterized network-level differences between subtypes and between ASD subtypes and TD individuals, and further showed that individuals within each subtype exhibit different relationships between FC metrics and a set of behavioural measures.

4.2. Static FC

Subtype 2 was defined by greater static FC within networks, particularly within the occipital network, and lower static FC between networks, especially between the DMN and other RSNs, compared to Subtype 1. Connections within networks tended to be positive on average in Subtype 2 and negative in Subtype 1, indicating reduced interactions among brain regions within these networks in Subtype 1. Further, connections between networks that were lower in Subtype 2 tended to be negative, but were positive on average in Subtype 1. As anti-correlations between resting-state networks are hypothesized to signify the division of labour between brain regions

that are involved in different functions (Fransson 2006), and the ability for regions that are relevant for certain cognitive functions to become activated with concurrent deactivation of irrelevant regions (Fox et al., 2005; Greicius et al., 2003), these abilities may be affected in Subtype 1.

A second LV showed that both subtypes could be differentiated from the TD group. More specifically, both subtypes exhibited reliable decreases in static FC within the SMN and DMN compared to controls, but showed greater static FC within the occipital network (Fig. 15). Atypical static FC of sensorimotor regions has reported in previous studies (Anderson et al., 2011a; Mostofsky et al., 2009; Turner et al., 2006). Our findings suggest that despite the broad range of sensorimotor difficulties experienced by individuals with ASD (Minshew et al., 1997; Perry et al., 2007; Whyatt & Craig, 2013), atypical functioning with the SMN may be common across the autism spectrum. The DMN has been a focus of many FC studies in those with ASD, particularly because it has been hypothesized that abnormal DMN functioning relates to decreased self-referential processing, a decreased ability to redirect attention from external to internal processing, and difficulties with theory of mind in ASD (e.g. Assaf et al., 2010). Many studies have reported decreased FC between DMN regions in those with ASD (Assaf et al., 2010; Kennedy & Courchesne, 2008; Monk et al., 2009; Weng et al., 2010), although hyperconnectivity within this network has also been reported (Monk et al., 2009; Uddin et al., 2013a). One study found that hypo- or hyperconnectivity of the DMN may be region-specific (Doyle-Thomas et al., 2015). Increased FC in the occipital network is consistent with previous findings of increased local connectivity in primary visual regions (Keown et al., 2013) and increased involvement of extrastriate cortex (Shen et al., 2012) in ASD. Further, reliably higher FC was found between the DMN and FPN, and between the CON and CER networks. These connections were positive on average in ASD, but negative on average in controls. Previous studies have also reported reduced negative connectivity in ASD, which was described as reduced functional segregation of networks (Rudie et al., 2012; 2013a) or underconnectivity of long-range inhibitory connections (Anderson et al., 2011b). Our results show that despite distinct differences in static FC between subtypes, atypical FC within certain networks is consistent across all ASD participants compared to controls.

4.3. Dynamic FC

Dynamic FC analyses indicated that Subtype 2 was characterized by greater temporal stability of dynamic FC compared to Subtype 1, which was most prominent within the occipital network, and was also evident in the analyses of the contributions of within- and between-network connections to the spatial patterns (Fig. 15). Network-level analyses for the second LV showed that positive BSRs (ASD > TD) were reliable within the FPN and OCC, and negative BSRs (TD > ASD) were reliable within the DMN and SMN. Decreased temporal stability, or increased variability, has been reported in recent studies of dynamic FC in individuals with ASD (Chen et al., 2017; Falahpour et al., 2016; Zhang et al., 2016). Chen et al. (2017) found a cluster of connections that were more variable in ASD, and were particularly prominent in prefrontal and temporal regions, especially the right medial superior frontal gyrus. Overall, these "hypervariant" connections tended to occur for long-range connections. Zhang et al. (2016) reported increased variability of connections involving primarily medial frontal regions. Falahpour et al. (2016) reported increased connection variability amongst DMN regions, specifically between the PCC and medial PFC, and between the medial PFC and lateral parietal cortex. Further, the investigators found that lower static FC in ASD was mediated by higher variability of FC; in other words, group differences in static FC were significantly impacted by group differences in temporal variability of FC. Future studies should examine the potential role of increased stability in the FPN and OCC networks in individuals with ASD.

It has been hypothesized that increased temporal variability, or decreased temporal stability, in ASD may signify more rapid transitions of mental states, or may render the brain less prepared for cognitive and sensorimotor processing (Falahpour et al., 2016). The finding of decreased temporal stability also relates to the hypothesis that ASD in characterized by a decreased signal to noise ratio (LeBlanc & Fagiolini, 2011; Minshew & Keller, 2010; Rubenstein et al., 2003). It has been theorized that an increased excitatory-inhibitory ratio in the brain is characteristic of ASD, which leads to poor functional differentiation of networks and noisier, more unstable signaling (Rubenstein & Merzenich, 2003). While some degree of noise is beneficial for information processing in the brain (McIntosh, Kovacevic, & Itier, 2008), too much physiological noise in the brain may be associated with a reduced ability to efficiently explore different network configurations (Winterer et al., 2000). Further, Chen et al. (2017) noted that increased variability of dynamic FC may disturb the processing of cognitive and social information in individuals with ASD. Interestingly, while there were reliable associations

between dynamic FC and behaviour in Subtype 2, these relationships were not reliable in Subtype 1. It could be hypothesized that individuals in Subtype 1, who exhibit more variable dynamic FC compared to those in Subtype 2, show more idiosyncratic relationships between dynamic FC and behaviour that reflect different mechanisms to compensate for decreased efficiency of information processing. Future studies should determine if these different brain-behaviour relationships between subtypes hold true during task performance.

4.4. Relationships between FC and behaviour

For static FC, reliable positive and negative correlations between FC and behaviour were observed both within and between several networks for Subtype 2. Dynamic FC analyses revealed that both positive and negative correlations were found between the occipital network and other networks, and the only reliable within-network correlations were found within the occipital network. Therefore, temporal stability within the occipital network may be an important modulator of ASD-related behaviours. Previous studies have reported mixed results regarding associations between FC measures and ASD behavioural measures. For instance, Keown et al. (2013) found that overconnectivity in posterior brain regions was associated with greater severity of ASD symptoms, and that frontal underconnectivity was found only in low-severity participants. However, another study found that ASD severity was correlated with the extent of hyperconnectivity in the salience network, which includes regions such as the dorsal anterior cingulate cortex and frontoinsular cortex (Uddin et al., 2013b). Lee et al. (2016) reported overall reduced FC density in ASD, and found that average interhemispheric FC density and contralateral FC density in a lingual/parahippocampal gyrus cluster and default mode network (DMN) regions was negatively correlated with RRBs. On the other hand, hyperconnectivity between the posterior cingulate cortex (PCC), a core region of the DMN, and the right parahippocampal gyrus was associated with more severe RRBs in another study (Monk et al., 2009). These investigators also reported that weaker FC between the PCC and superior frontal gyrus was correlated with reduced social functioning in ASD participants. One study found that idiosyncratic distortions in FC related to ASD symptom severity, whereby greater distortions from a typical canonical template of FC were associated with greater symptom severity as measured by the ADOS (Hahamy et al., 2015). Recent studies of relationships between dynamic FC and ASD behaviours reported positive correlations between temporal variability and ADOS

total scores, with most connections involving the right medial superior frontal gyrus and left middle temporal pole (Chen et al., 2017), and positive correlations between temporal variability in several DMN regions and RRB scores (Zhang et al., 2016). Crucially, we did not find reliable relationships between FC and behaviour when all ASD participants were considered as a single group, which reveals the importance of defining subtypes of ASD in order to elucidate relationships between FC and behavioural measures. However, while our results suggest the importance of the degree of temporal stability within the occipital network in the relationship between FC and behaviour in the second ASD subtype, global patterns of correlations between static FC and behaviour were less clear. In addition, we did not observe reliable correlations between FC and behaviour for connections within the DMN, as was observed in recent studies. Future studies should consider defining a greater number of ASD subtypes to determine whether clearer patterns of correlations between FC and behaviour arise.

4.5. Relationships between static and dynamic FC

Correlations of the brain saliences between static and dynamic FC for PLS-MC and PLS-B revealed a strong relationship between these two FC patterns. It has been demonstrated previously that dynamic FC is related to static FC in that weaker static connections exhibit greater temporal variability (e.g. Betzel et al., 2016; Falahpour et al., 2016; Li et al., 2015; Thompson & Fransson, 2015). Nonetheless, analyses of overlapping and unique static and dynamic connections showed that dynamic metrics provides information about FC that is not provided by static metrics alone. As shown in Fig. 16, overlapping static and dynamic connections were reliable in the DMN and SMN for both PLS-MC LV1 and LV2. Fig. 15 shows that connections within these networks were reliably lower in Subtype 1 compared to Subtype 2, and in all ASD participants relative to controls. These findings again suggest the possibility that reduced communication and/or reduced temporal stability within these networks may be a more robust finding across the autism spectrum, although may be more prominent in individuals with FC patterns that are more similar to Subtype 1 compared to Subtype 2.

4.6. Other characteristics of ASD subtypes

It is interesting to note that the ASD subtypes did not significantly differ in terms of IQ or ADOS severity scores, presence of comorbidities, medication use, or eye status. This finding is

important because it reveals that meaningful brain-based subtypes of ASD are not simply based on high and low behavioural severity. In other words, there is utility in defining subtypes based on FC metrics and not simply using behavioural measures to define these subtypes.

4.7. Limitations

It is important to note that it was necessary to control for age, head motion, and scan site when defining the two ASD subtypes; when these variables were not controlled for, there were significant differences in these variables either between subtypes or between at least one subtype and controls. This is particularly concerning for head motion, because it shows that despite regression of motion parameters before extracting fMRI time series, there are still residual effects of head motion that are evident in the FC matrices. This phenomenon has been documented previously (e.g. Power et al., 2012), and several strategies have been proposed to mitigate these effects (Power et al., 2014). Future studies should focus on further elucidating the effects of head motion on FC, and determine which motion correction strategies best control for these effects. It is possible that more rigorous approaches for removing the effects of motion from fMRI time series may prevent the need to regress residual motion effects from FC matrices when defining FC-based subtypes of ASD to ensure that motion parameters do not differ between subtypes.

Another limitation of our study is that we defined subtypes using a single preprocessing strategy. While defining subtypes of ASD based on FC may help to resolve some discrepancies in the literature regarding the nature of FC in ASD, it has been proposed that differences in analysis approaches between studies are the most likely causes of inconsistent results between studies of FC in ASD (Hull et al., 2016). For instance, Muller et al. (2011) found that studies supporting the “general underconnectivity” hypothesis of ASD were more likely to not use low-pass filtering and to utilize ROIs as opposed to whole-brain analyses. It has also been reported that removal of the global signal alters group differences in FC observed between participants with ASD and TD participants (Gotts et al., 2013). Therefore, it is crucial to gain a more thorough understanding of how a variety of preprocessing choices affect observed group differences in FC, and to compare FC-based subtypes of ASD across different preprocessing strategies.

An additional limitation relates to the unreliable relationships between FC and behaviour in Subtype 1. It is important to consider that the total scores on the ADOS and ADI do not fully capture the heterogeneity of the types of behaviours that can be displayed by those with ASD. It could be that different types of behaviours, for instance, simple repetitive motor behaviours versus higher order repetitive behaviours such as insistence on sameness, would be associated with different atypical patterns of FC in large-scale networks. Thus, future studies should examine relationships between more specific behaviours and FC patterns in FC-based subtypes of ASD. This may reveal more specific relationships between FC and behaviour in Subtype 1 in particular. Further, we only defined two subtypes of ASD, as this was the ideal number of subtypes based on the elbow criterion. However, it is possible that characterizing a greater number of subtypes may reveal more nuanced relationships between FC and behaviour in each subtype.

4.8. Conclusions

This study reveals the importance of considering brain-based subtypes of ASD, which have the potential to provide insight into different relationships between FC and behaviour relative to controls. Brain-based subtypes of ASD may help to cater treatments and behavioural therapies to different subtypes. This work also highlights the importance of utilizing both static and dynamic FC when characterizing ASD; while the spatial patterns for these metrics were somewhat correlated, each metric also provided unique information about connections' contributions to the patterns. Static and dynamic FC metrics, combined with multivariate statistical approaches, will be important for further characterizing the nature and development of FC in individuals with ASD.

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