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The Default Network and Autism Spectrum Disorder: Characterizing Sub-Networks and Behavioral Correlates

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ABSTRACT

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The default network (DN), and specifically its sub-networks default network A (DN A) and default network B (DN B), has been strongly implicated in social cognition. This study examined its role in predicting social behavior, and also differences that may exist across diagnostic groups that may explain discrepancies in social cognition and behavior. One of the popular methods of study is functional connectivity, or analyzing correlated activity in the brain. Autism Spectrum Disorder (ASD) is a disorder characterized by social impairment and abnormal social behavior. To date, much of the functional connectivity research in ASD has focused on global connectivity, or specific but large areas of the brain. This study adds to the body of that research in attempting to understand both global functional connectivity and the functional connectivity of specific networks (DN A and DN B) that are involved in social cognition and thus implicated in ASD. A sample of 75 individuals with ASD, 85 neurotypical individuals, and 505 individuals with varying other diagnoses was examined to determine the role of global functional connectivity and the role of DN A and DN B in social cognition by the predictive ability of brain features to determine behavioral outcomes. This analysis also aimed to determine if there are group differences in these same brain features. The features we examined included functional connectivity, or the comparison of timeseries of regions of interest, network surface area, and network similarity. This study found that there was no discernible difference across diagnostic group in global or network-specific functional connectivity for DN A. The majority of features for DN B did not differ across diagnostic group, but there was one connection that was significantly different between the autism group and the others. There was no global predictive ability of functional connectivity and brain topology for social cognition measures, nor was there predictive ability for DN A features. DN B features, however, were predictive of social cognition in the autism group, but not in the control group or the other diagnostic groups examined. This study adds to the current body of research by supporting findings already reported by others, and by adding new findings about the role of DN B in social cognition in autism.

Keywords: autism, functional connectivity, default network, brain organization, development

TABLE OF CONTENTS

LIST OF FIGURES

Introduction

Autism Spectrum Disorder (ASD) is a developmental disorder that affects a relatively large proportion of the population of the world, as many as 1 in 54 children (Centers for Disease Control and Prevention, 2020). It is characterized by several distinct symptoms and behaviors, including socio-communicative impairments as well as cognitive impairments and repetitive or restrictive behaviors, and has most often been studied in children (Lord et al., 2018; Kanner, 1943). These symptoms occur on a spectrum of severity, as the name of the disorder implies, and can severely impact autistic childrens' quality of life. Those with severe symptoms require near-constant care, while others live more independently (CDC, 2020).

Autistic children often experience a diminished quality of life due to symptoms of varying severity and attempted coping mechanisms (Griffiths et al., 2019). Some of the symptoms that can disrupt quality of life are increased sensory sensitivity (Cerliani et al., 2015), uncontrollable or repetitive movements or behaviors (Brodski-Guerniero et al., 2018), reduced cognitive ability (Lynch et al., 2017), and reduced communication ability (Lynch et al., 2013; Murdaugh et al., 2012). Each of these symptoms has a spectrum of severity and impact unique to each individual (Cerliani et al., 2015; Jann et al., 2015).

Mechanisms and Neural Abnormalities

The biological mechanisms of ASD are not well understood; it is accepted that neural abnormalities exist, but the major underlying causes are largely unknown (Ecker et al., 2015). Studies show differences begin early in life, and if characterized are accurate predictors of an ASD diagnosis later in childhood: indicators at 6-12 months of age predict a diagnosis at 2-3 years of age (Emerson et al., 2017; Hazlett et al., 2017; Shen & Piven, 2017). Eye tracking

studies have shown an observable difference in social face preference very early on that correlate strongly with later diagnosis of ASD (Jones & Klin, 2014).

While some siblings of autistic children do not express the symptoms of the disorder, siblings are at higher risk to develop ASD, highlighting the heritability and genetic component of autism (Constantino et al., 2006; Bailey et al., 1998). Some abnormalities that occur in unaffected siblings to a lesser degree show that these siblings can potentially experience a broader ASD phenotype without receiving a diagnosis of ASD (Moseley et al., 2015; Spencer et al., 2012; Pisula & Ziegart-Sadowska, 2015).

Some of the neural differences that have been studied in ASD are incredibly variable across individuals and are often hard to reproduce (King et al., 2019). Some reasons for the lack of reliable biological differences include the heterogeneous causes of ASD, diversity in presentation and severity of symptoms, small sample size, lack of longitudinal data or cross-sectional data at consistent ages (childhood vs adulthood), lack of sufficient scanning time for each individual, and others (King et al., 2019; Abbott et al., 2016; Nielsen et al., 2013). Findings have varied depending on sample size, imaging techniques, networks, areas of study, and in autism, severity of diagnosis (Abbott et al., 2016; King et al., 2019; Nielsen et al., 2013; O'Reilly et al., 2017). Significantly, Müller and colleagues showed that findings have also varied depending on methods of data collection and analysis (Müller et al., 2011). This includes variation in type and method of imaging, inclusion or exclusion criteria for participants, and analysis techniques as previously described. The variation inherent in previous studies has made replication difficult, and thus defining functional connectivity in the context of previous research is not reliably possible (Müller et al., 2011). There are some abnormalities that have been fairly consistent and reproducible across age, sex, and severity of diagnosis, though there have been

contradictory findings even in these (Müller et al., 2011). They include anatomical abnormalities such as reduced cortical thickness, reduced gray matter volume, increased total brain volume, and hyperextension of cortical surface area, and each has been seen through differing stages of development (Ecker et al., 2015; Hazlett et al., 2017; Pereira et al., 2018), and connectivity abnormalities, although findings in this area have remained inconsistent in the particulars (Bernas et al., 2018; Ecker et al., 2015; Gao et al., 2019; Jann et al., 2015; Keown et al., 2017; Wang et al., 2018). Abnormalities in both structure and connectivity discovered in autistic individuals have the potential to increase understanding of the mechanisms behind ASD. Studying brain networks in particular has the potential to broaden understanding by correlating relationships between what is seen in the presentation of symptoms and what is happening within the brain.

Methods of Investigating Functional Connectivity

Many techniques and approaches to studying both structural and functional connectivity have been used, including diffusion tensor imaging (DTI) to look at structural connectivity and electroencephalography (EEG) and magnetoencephalography (MEG) to examine functional connectivity (Greicius et al., 2009; O'Reilly et al., 2017; Zeng et al., 2017). Functional connectivity in particular has often been used to study brain network organization and correlation. This method uses functional neuroimaging and/or electrophysiology to characterize connectivity between brain areas based on correlated brain activity (Du et al., 2018). This correlated activity has been postulated to underlie structural connections that can potentially be identified using the observed functional connections (Barbas, 2015). It has been used to identify brain network abnormalities in a variety of psychiatric and developmental disorders, including ASD (Barbas, 2015).

Another popular neuroimaging method that applies functional connectivity analyses to functional magnetic resonance imaging (fMRI) is resting-state functional magnetic resonance imaging (rs-fMRI) (Kong et al., 2019; Yeo et al., 2011). Advantages to rs-fMRI include the ability to obtain a resting baseline and the lack of a task completion requirement (Smitha et al., 2017). One other advantage that is enhanced by rs-fMRI is a longer scanning time, which allows for more robust analysis (Nielsen et al., 2013; Smitha et al., 2017).

Analysis of functional connectivity data varies widely between studies. Previously, several approaches have been taken in analyzing data associated with both global and network-specific functional connectivity. Among them are the complex network analysis (Moseley et al., 2015; Yerys et al., 2015), seed-based analysis (Abbott et al., 2016; Braga et al., 2019; DiNicola et al., 2020; Gabrielsen et al., 2018; Gao et al., 2019; Greicius et al., 2009; Joshi et al., 2017; Jung et al., 2015; Murdaugh et al., 2012; Redcay et al., 2013; von dem Hagen et al., 2013), and independent components analysis (Assaf et al., 2010; Cerliani et al., 2015; Hyatt et al., 2020; Jung et al., 2015; von dem Hagen et al., 2013; Weng et al., 2010). Yeo and colleagues revolutionized the methods to study functional connectivity using rs-fMRI with their methods of creating connectivity profiles, combining the description of networks with a clustering algorithm and using a very large dataset (n = 1000); many studies have built on their foundation and continue to do so (Yeo et al, 2011).

Functional connectivity analysis aims to understand brain function by observing temporal correlation of activity in a resting state and using that to infer connections in the brain defined as networks (Chen & Glover, 2015). High connectivity indicates high correlated activity as shown on an fMRI scan, within or between networks; low connectivity is defined similarly, with a corresponding lower level of correlated activity (Du et al., 2018).

Functional Connectivity in Autism

Functional connectivity analysis has been highly prevalent in more recent studies on ASD (Nair et al., 2020). Using a variety of analysis techniques, many studies have found decreases in connectivity in ASD both globally and in specific areas or networks, including the default network (DN), salience network (SN), and dorsal attention network (DAN), among others (Cheng et al., 2015; Guo et al., 2019; Joshi et al., 2017; Nair et al., 2020; von dem Hagen et al., 2013; Weng et al., 2010). Reduced connectivity has been seen on a global or whole-brain level (Bernas et al., 2018; Moseley et al., 2015; Murdaugh et al., 2012; Zeng et al., 2017; Tang et al., 2020), though meta-analysis has shown that findings of global hypoconnectivity greatly depend on analysis methods (Müller et al., 2011). Although decreased connectivity has been a common finding, increased connectivity has also prevailed as the main finding in some studies, contradictory to other results (Cerliani et al., 2015; Keown et al., 2017; Jann et al., 2015). Specific areas have included sensory areas and hubs of connectivity with connections to several different networks (Cerliani et al., 2015; Keown et al., 2017).

In addition to studies that have found increased or decreased connectivity, there are studies that have found both types of abnormalities existing in autistic individuals (Di Martino et al., 2014; Hahamy et al., 2015; Lynch et al., 2013; Maximo et al., 2013; Pereira et al., 2018; Tang et al., 2020; Wang et al., 2018; Yerys et al., 2015). Specified networks and areas with decreased connectivity include anterior cingulate cortex (ACC), anterior DN, entire DN, and posterior cingulate cortex (PCC) (Lynch et al., 2013; Maximo et al., 2013; Pereira et al., 2018; Yerys et al., 2015). Specific areas linked to increased connectivity have included PCC, retro-splenial cortex (RSC), visual cortex, anteromedial prefrontal cortex (amPFC), sensorimotor regions of the DN, and the DN in general (Lynch et al., 2013; Maximo et al., 2013; Pereira et al.,

2018; Yerys et al., 2015). Both global reduced connectivity and global increased connectivity have been reported in several datasets as well (Di Martino et al., 2014; Hahamy et al., 2015; Tang et al., 2020; Wang et al., 2018). Proposed reasoning for these conflicting findings includes idiosyncrasy in functional connectivity across individuals, along with other hypotheses (Hahamy et al., 2015). However, though this topic has been studied in several different ways, findings remain conflicted (Abbott et al., 2016; King et al., 2019; Nielsen et al., 2013; O'Reilly et al., 2017).

Some studies making inquiries about ASD and functional connectivity are more specific than global connectivity or connectivity in different networks. Findings examining interactions between networks have found evidence of reduced integration and segregation (Joshi et al., 2017; Keown et al., 2017), along with both increased and decreased connectivity between specific networks (Gabrielsen et al., 2018; Gao et al., 2019; Kernbach et al., 2018; Plitt et al., 2015) or specific areas (Guo et al., 2019). Between-network studies have revealed many abnormalities in connections, adding to the conflicting literature cited previously (Andrews-Hanna et al., 2014; Cerliani et al., 2015; Cheng et al., 2015; Gabrielsen et al., 2018; Kernbach et al., 2018; von dem Hagen et al., 2013). Some researchers have studied local connectivity, meaning connectivity between neighboring neurons and white matter tracts, finding decreased connectivity in some areas and increased connectivity in others (Dajani & Uddin, 2016; Guo et al., 2019; Maximo et al., 2013; O'Reilly et al., 2017). Connections within networks have been found to be important, along with connections between networks; Gabrielsen and colleagues (2018) found decreased within-network connectivity in the DN, SN and other networks, and decreased interhemispheric connectivity.

Functional connectivity studies about ASD have found that often there are correlations between connectivity abnormalities and the severity of observable symptoms (Dajani & Uddin, 2016; Gao et al., 2019; Jann et al., 2015; Plitt et al., 2015; Redcay et al., 2013; Weng et al., 2010). This has, to some degree, been expected and interpreted to mean that ASD deficits and symptom presentations occur on a continuum (Ympa et al., 2016). Some associations have been very specific, such as increasing connectivity in anterior DN being associated with severity of social impairments (Jann et al., 2015), while others are more general, simply stating a correlation such as higher local connectivity in regional homogeneity—a comparison of a voxel's timeseries to its nearest neighbors—means more severe symptoms (Dajani & Uddin, 2016). Occasionally, with some symptoms such as language, no correlations have been found between connectivity and symptom severity, but this is still disputed (Pereira et al., 2018).

The Default Network

The DN encompasses areas of the brain largely in the medial frontal, lateral and medial parietal, and medial and lateral temporal cortices (Raichle, 2015). Among connectivity studies, the DN has been one of the networks that is the most studied in autism, as it has much to do with social and cognitive functioning (Nair et al., 2020). It has been observed to be involved in tasks such as introspection or mind wandering and mentalizing, among other activities (Gordon et al., 2020; Hyatt et al., 2020; Padmanabhan et al., 2017). Within many networks, but specifically larger networks such as the DN, there are centers of activity that have been identified as "hubs" that have also shown both reduced and increased activity (Redcay et al., 2013). Hubs, loosely defined, can include areas of high activity within a pathway, or areas of high correlated activity from several pathways (Power et al., 2013).

The DN was originally discovered and defined as brain areas that were less active or "deactivated" during task conditions and active at rest, however it is active during many activities and states of the brain (Buckner et al., 2008; Golland et al., 2007; Greicius et al., 2003; Raichle, 2015). Hubs, or areas with high density connections and larger amounts of activity, were characterized within the DN as more was learned about it and its role in executive functioning and cognitive control (Power et al., 2013). Additionally, roles for the DN have continued to emerge as more is learned, including the theory of the DN as an integration network (Yeshurun et al., 2012). After a few years studying and characterizing the DN with rs-fMRI, it has been found to be significantly involved in social cognition, which points to a possible association of the DN in ASD (Mars et al., 2012). Recent discoveries have included updated, more specific anatomy of the DN (Buckner & DiNicola, 2019) and as research techniques have improved, the DN has been divided into distinct and observable sub-networks (Andrews-Hanna et al., 2014; Braga et al., 2019; Braga & Buckner, 2017).

Sub-networks are smaller networks within a larger network that sometimes can be distinguished by function or correlation to the overall functioning of the larger network (Braga et al., 2019; Braga & Buckner, 2017). Studies of the DN have defined between two and nine sub-networks, though findings sometimes differ on how many independent sub-networks are definable (Andrews-Hanna et al., 2014; Braga et al., 2019; Braga & Buckner, 2017; DiNicola et al., 2020). Currently the most robust findings implicate two major sub-networks, default network A (DN A) and default network B (DN B) (Braga & Buckner, 2017; Braga et al., 2019). Notably, these sub-networks have been visible in multiple individuals, but only when the analysis is at an individual level rather than a group level (Braga et al., 2019).

Each of the two main sub-networks has been shown to be involved in differing function in the DN, with DN A being involved in episodic projection and DN B in social cognition and Theory of Mind (ToM) (DiNicola et al., 2020). The two networks are interdigitated, with parallel areas in the frontal, parietal, and temporal lobes, and thus difficult to differentiate in a group averaged parcellation of the brain (Figure 1) (Braga & Buckner, 2017; Braga et al., 2019). As mentioned before, however, when observed in individuals, DN A and DN B can be consistently observed in each individual, with the inherent variation in all brain structures. When observed during task conditions, DN A and DN B have manifested separate and distinct functions, even though they work together as part of a larger network (DiNicola et al., 2020).

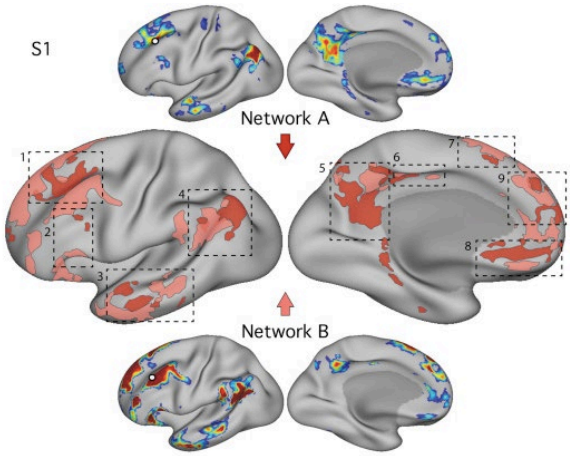

Figure 1: Hypothesized interdigitated default network sub-networks A and B (Braga & Buckner, 2017).

The DN has long been implicated both in executive function and cognitive processing (Andrews-Hanna et al., 2010; Raichle, 2015; Woolnough et al., 2020). Functions include those attributed to dorsomedial prefrontal cortex (dmPFC) (Jamali et al., 2021) and involved in ToM, which is the ability to attribute emotional states and reactions to oneself and others (Cheng et al., 2015; Murdaugh et al., 2012; Padmanabhan et al., 2017). Additionally, functions of the DN include those involved in social interaction (Mars et al., 2012; Raichle, 2015) as well as

mentalizing and internal cognition (Andrews-Hanna et al., 2014; Buckner et al., 2008; DiNicola et al., 2020; Hyatt et al., 2020; Padmanabhan et al., 2017).

The Default Network, Cognition, and Autism

Understanding that the DN is involved in cognitive processing, specifically in mental exploration and related processes, there is significant potential for altered DN activity to reflect differing cognitive processing (Kong et al., 2019). A recent study showed that connectivity can be predictive of behavioral measures; this leads to the thought process that alterations in connectivity or network activity may have an effect on behaviors associated with that particular network or brain area (Kong et al., 2019). Studies of ASD have shown symptoms indicative of these alterations, including deficits in social and communication strategies and in language processing (Lord et al., 2018). There is reason to suspect that the DN is involved in ASD because of the correlation with functions of the DN such as mentalizing, internal cognition, and social interaction as mentioned above and the major presenting symptoms of ASD at diagnosis and throughout life (Mars et al., 2012; Raichle, 2015; DiNicola et al., 2020; Padmanabhan et al., 2017; Lord et al. 2018). There is a growing body of evidence that functional connectivity is altered in ASD, and that there are observable symptoms of these alterations, but as mentioned previously, findings remain inconsistent (Abbott et al., 2016; King et al., 2019; Nielsen et al., 2013; O'Reilly et al., 2017). Consistent reproducibility has been found only with large sample sizes (King et al., 2019), and longer imaging time has been associated with better accuracy (Nielsen et al., 2013). It is known that there are abnormalities in ASD specifically in the DN (Assaf et al., 2010; Joshi et al., 2017; Padmanabhan et al., 2017; Pereira et al., 2018), and that abnormalities in behavior have been predictable by differences in structure and function, both in autistic and typical individuals (Greicius et al., 2009; Kong et al., 2019; Pereira et al., 2018).

This study is designed to broaden the understanding of the role of the DN and its sub-networks in ASD and discover if a robust correlation can be found between connectivity abnormalities and behavior. Previous conflicting findings have made it difficult to establish a clear role for the DN in ASD, and many of the previous studies have varied both in technique and data collection design. One substantial difficulty with studies about ASD is sample size—many of the previous studies about the role of the DN in ASD have had significantly restricted sample sizes. New, larger datasets have begun to alleviate this difficulty, and will going forward allow studies to elucidate more robust correlations and associations. To date, there are no connectivity studies that attempt to discern between DN sub-networks and associate them with connectivity abnormalities in ASD and how they associate with behavior. This study uses an individualized approach to characterize sub-networks that allows for a clearer view of what has previously been viewed as a single network.

Aims

Aim 1

The first aim of the study is to draw parallels from functional connectivity to observed autistic behavior.

Aim 1.1

Considering previous findings, we hypothesize that functional connectivity in the DN and specifically in DN B would be reduced in an autism sample compared to other diagnostic groups.

Aim 1.2

This study will analyze the predictive ability of all whole-brain functional connectivity and brain topology features for measures of social cognition. Globally, we hypothesize that

functional connectivity and brain topology will only be able to predict performance on behavioral tests with little to no accuracy.

Aim 1.3

This study will also analyze the predictive ability of functional connectivity and brain topology specific to DN A and DN B. We hypothesize that functional connectivity and brain topology will be able to predict social outcomes in DN B, but not in DN A. We hypothesize that this predictive ability will be consistent across diagnostic group.

Aim 2

The second aim of this study is to differentiate connectivity abnormalities across diagnostic groups in the whole brain and in the major sub-networks of the DN, defined in recent studies as DN A and DN B (Braga & Buckner, 2017; Braga et al., 2019).

Aim 2.1

This study will compare global functional connectivity features across diagnostic group. It is expected that there will be little or no effect seen globally in an autism sample versus other groups.

Aim 2.2

This study will compare functional connectivity features in DN A and DN B across diagnostic group. It is expected that DN A will have no discernable differences across diagnostic group, but that DN B would show reduced connectivity because of its involvement in social cognition and ToM (DiNicola et al., 2020). Because DN B is involved in social cognition, we hypothesize that these differences in connectivity will be specifically shown in DN B in the autism sample when compared to other groups.

Aim 2.3

This study will examine measures of topology in the brain across diagnostic group, both globally and specifically in DN A and DN B. We hypothesize that measures of topology will not differ globally, but in DN B will be different in the autistic population when compared with other groups.

Methods

Overview

In this project, I closely followed methods outlined by Kong and colleagues, which analyzed how spatial topography and functional connectivity of brain networks correlate with cognition and personality (Kong et al., 2019). I have applied these methods to a population of individuals with an ASD diagnosis in order to understand whether features of connectivity in the default network (DN) and its sub-networks (DN A and DN B) significantly differ compared to typical individuals.

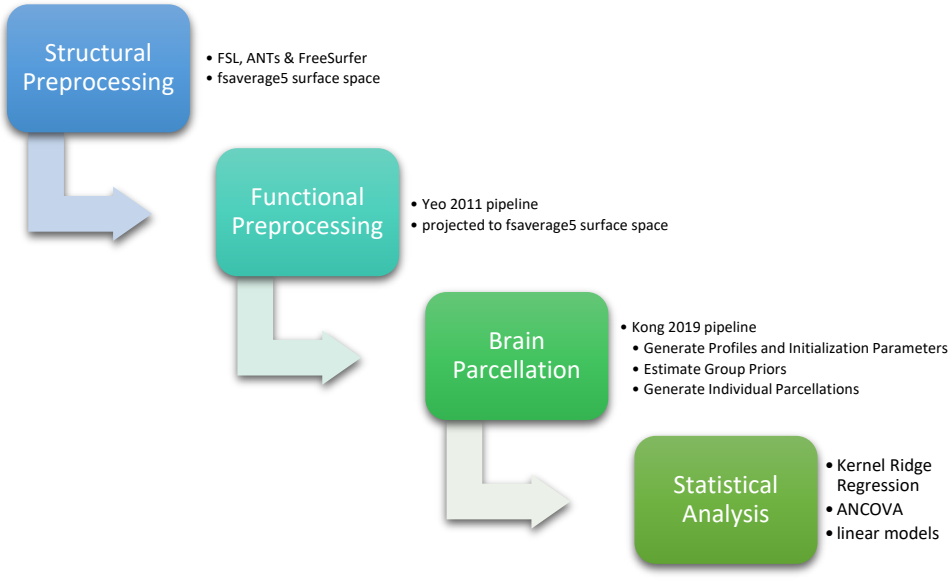

Figure 2: Outline of methods used in this study. Details provided in sections below, and scripts are found on GitHub (https://github.com/ThomasYeoLab/CBIG).

Healthy Brain Network Dataset

The Healthy Brain Network (HBN) dataset (http://fcon_1000.projects.nitrc.org/indi/cmi_healthy_brain_network) is made up of imaging and behavioral data gathered from 3625 individuals with varying diagnoses, 2331 of them male, 1294 female. Mean age for subjects in this dataset is 10.52 ± 3.67 years. Five hundred-fifty of these individuals have a diagnosis of ASD, and 756 have no diagnosis given. Other individuals in the dataset have varying diagnoses, such as anxiety disorders, attention deficit hyperactive disorder (ADHD), communication disorders, or learning disorders, among others (Alexander et al. 2017). Participants were recruited using a community-referred recruitment model.

Inclusion criteria for the HBN dataset included being male or female, ages 5-21 years, the capacity to provide assent (if under the age of 18) or informed consent (18+), and fluency in the English language. Children must have had the ability to give verbal affirmation at a kindergarten level, and the parent or guardian must have been capable of providing informed consent. If children were bilingual and fluent in English but had a parent that spoke only Spanish, they were accommodated. Exclusion criteria included acute encephalopathy due to injury or disease, known neurodegenerative disorder, hearing or visual impairment that was uncorrected by hearing or visual devices and prevented completion of tasks, recent diagnosis of schizophrenia, schizoaffective disorder, or bipolar disorder without treatment, acute manic or psychotic episode without current and ongoing treatment, new onset of suicidality or homicidality without treatment, history of lifetime substance dependence requiring chemical replacement therapy, or acute intoxication at the time of data collection (Alexander et al., 2017).

Data collected included diagnostic evaluation, phenotypic assessment, cognitive task assessment, electroencephalograpy (EEG) and multi-session magnetic resonance imaging (MRI).

The data that will be used for the current study will be the diagnostic evaluation, phenotypic assessment, and cognitive evaluation assessment, as well as structural and functional MRI data.

MRI Parameters

Early MRI data were collected using a 1.5 T Siemens Avanto system, with 45mT/m gradients in a mobile trailer (Alexander et al., 2017). For stability, the trailer was parked on 10-inch thick concrete pads. The system included 32 RF receive channels, a Siemens 32-channel head coil, and the University of Minnesota Center for Magnetic Resonance Research (CMRR) simultaneous multi-slice echo planar imaging sequence. Each scan included resting state functional MRI (fMRI), diffusion kurtosis imaging (DKI), structural MRI (T1-weighted and T2-weighted), magnetization transfer imaging, quantitative T1 and T2 mapping (DESPOT T1/T2), and imaging of visceral fat (T1W).

Later scanning, which included the majority of the data, was done using a 3T Siemens scanner, with a standard 32 channel head coil and the CMRR simultaneous multi-slice echo planar imaging sequence (Alexander et al., 2017). Participants engaged in resting state scans, as well as a visual task watching a short movie.

There were two locations of data collection, Staten Island, New York, and Rutgers University. At Staten Island, parameters of the data used in the current study were as follows: T1 MPRAGE (176 slices; %FOV phase = 100%; resolution = 1.0 mm x 1.0 mm x 1.0 mm; TR = 2730 ms; TE = 1.64 ms; TI = 1000 ms; flip angle = 7°) and fMRI (54 slices; %FOV phase = 100%; resolution = 2.0 mm x 2.0 mm x 2.0 mm; TR = 1450 ms; TE = 40 ms;flip angle = 55°). At Rutgers University, parameters were as follows: T1 MPRAGE (224 slices; %FOV phase = 100%; resolution = 0.8 mm x 0.8 mm x 0.8 mm; TR = 2500 ms; TE = 3.15 ms; TI = 1060 ms;

flip angle = 8°) and fMRI (60 slices; %FOV phase = 100%; TR = 800 ms; TE = 30 ms; flip angle = 31°).

Diagnostic Evaluation

All diagnostic assessments and evaluations were administered by licensed clinicians (Alexander et al., 2017). The first was what the authors term a semi-structured diagnostic interview; each participant was given a web version of the Schedule for Affective Disorders and Schizophrenia—Children's Version (KSADS) (Kaufman et al., 1997). This is a DSM-5-based interview used to pinpoint clinical diagnoses in patients and was performed by a licensed clinician. This included a parent interview and a child interview, then based on results of the interviews automated diagnoses were assigned. Following automated diagnoses, they were confirmed and synthesized by the clinical team; the data for each participant includes both the automated diagnoses and the diagnoses from the clinical team (Alexander et al., 2017).

Supplemental diagnostic assessment was done for subsets of disorder such as ASD, in which case the Autism Diagnostic Observation Schedule was performed (Alexander et al., 2017; Lord et al., 2012). Other suspected disorders were given diagnostic assessments corresponding with the disorder there was reason to suspect, as listed in Alexander et al. (2017). Additional diagnostic assessments were not given to any where there was no relevant suspicion of a clinically significant illness.

Phenotypic Assessment

Phenotypic assessment included general information as well as physical fitness information (Alexander et al., 2017). General information collected about each individual included demographics and handedness, as well as a physical activity questionnaire, a medical history questionnaire, a financial support questionnaire, and the Barratt Simplified Measure of

Social Status. Physical fitness information included tests of strength, cardiovascular fitness, vitals such as heart rate and blood pressure, measurements of height, weight, and waist circumference, a blood draw with endocrine, immunologic and metabolic profiling, blood draw and buccal swabs for genetic sequencing, urine samples for toxicology screening and pregnancy tests, a color vision test, and a sleep disturbance scale (Alexander et al., 2017).

Cognitive Evaluation

Participants ages 6-17 completed the Wechsler Intelligence Scale for Children (WISC-V) (Wechsler, 2003); participants ages 5 and those believed to have an IQ below 70 completed the Kaufman Brief Intelligence Test (KBIT) (Kaufman et al., 2013). Participants ages 18 and older completed the Wechsler Adult Intelligence Scale (WAIS-IV) (Wechsler, 2008), and all participants over the age of 6 completed the Wechsler Individual Achievement Test (WIAT III) (Test, 2005). Other cognitive tasks included in the imaging and other assessments included the NIH Flanker, Card Sort, and Processing Speed Temporal Discounting Task, the Rapid Automatic Naming and Rapid Alternating Stimulus Tests (participants age 5), the Differential Ability Scales—II (age 5 or IQ below 70), as well as language assessment tests (Alexander et al., 2017).

Behavioral Measures

Behavioral measures included a number of standardized tests and reports, some specific to individual disorders, but most administered to a large group of participants. These were meant to assess behavioral characteristics according to age at the time of testing, and included the Extended Strengths and Weaknesses Assessment of Normal Behavior (E-SWAN) (ages 5–17), the Repetitive Behavior Scale (RBS) (ages 5–21), the Autism Spectrum Screening Questionnaire (ASSQ) (ages 5+), the Social Communication Questionnaire (SCQ) (ages 5+), the Social

Responsiveness Scale-2 (SRS-2) (ages 5+), the Strengths and Difficulties Questionnaire (ages 5+), the Social Aptitudes Scale (SAS) (ages 5+), and others (Alexander et al., 2017).

Preprocessing

Structural Preprocessing

Structural preprocessing was done using a combination of FSL 6.0.5 to generate and convert files and ANTs 2.1.1 to correct for field bias, but the largest portion of the preprocessing was completed using FreeSurfer.

FreeSurfer version 7.1.1 was used to process the structural MRI data. This software package generates surfaces of the cerebral cortex from T1-weighted MRI scans (Dale et al., 1999). The two surfaces include one that estimates the white matter/gray matter boundary and the other the pia mater/cerebrospinal fluid boundary. The functional images are then projected to the surfaces generated by FreeSurfer (Dale et al., 1999). The entire FreeSurfer pipeline includes (1) correcting for magnetic resonance inhomogeneities (Dale et al., 1999), (2)"skull-stripping" or eliminating extra-cerebral voxels (Ségonne et al., 2004), (3) segmenting voxels by gray and white matter according to differences in intensity and geometric structure, (4) computing planes to segment the hemispheres and subcortical structures, (5) filling interior holes using connected-component analysis, (6) tessellating and then smoothing triangular mesh to produce a smooth cortical surface image with definition between structures (Dale et al., 1999), and (7) correcting topological abnormalities in the surface so the topology is roughly spherical (Fischl et al., 2001; Ségonne et al., 2007).

Functional Preprocessing and Alignment

The functional preprocessing followed a pipeline created by Yeo and colleagues (Yeo et al., 2011; Holmes et al., 2015), using Freesurfer (Dale et al., 1999; Fischl et al., 1999a; Fischl et

al., 1999b; Fischl et al., 2001; Ségonne et al., 2007; Greve & Fischl, 2009) and FSL (Jenkinson et al., 2002; Smith et al., 2004). As noted above, the preprocessed data is projected to the Freesurfer fsaverage5 surface space.

To begin the process, the first six volumes of each run were discarded to compensate for equilibration effects. After this, FSL 6.0.5 was used to correct for head motion (Smith et al., 2004). Further preprocessing was done using methods adapted from Biswal et al. (1995) that have been optimized by others for functional connectivity analysis (Fox et al., 2005; Van Dijk et al., 2010; Vincent et al., 2006). Constant offset and linear trend for each run was removed and a temporal filter was applied to retain frequencies below 0.08 Hz. Using linear regression, we corrected for head motion (as mentioned above), whole-brain average signal, signal averaged over the ventricles, and signal averaged over deep cerebral white matter (Yeo et al., 2011). This procedure also minimized noise from non-neural sources such as respiration and heart rate (Van Dijk et al., 2010).

Using FreeSurfer, after minimizing geometric distortion, a spherical coordinate system was assigned and functional data were overlaid on the same coordinate system as the structural data (Yeo et al., 2011). A 6mm full-width half-maximum smoothing kernel was then applied to the fMRI data and was then projected onto the structural image.

Quality Control and Motion Metrics

Structural quality control was done by visual inspection. Raw T1-weighted images were assessed for quality and the presence of motion artifacts, using a three-category rating system (Backhausen et al., 2016). Output images from Auto EACSF were assessed for quality of segmentation using the rating scale and standard images developed by (Murphy et al., 2020). Only subjects with a score of less than two (no abnormalities in segmentation or minor under- or

overestimation of EA-CSF in one region) and a T1-weighted image with a score less than three were included in this study (Murphy et al., 2020). The raters were blinded to the identities of the images and the overall interrater reliability was 0.95 before discrepancies in ratings were resolved. If raters differed in their ratings, a third rater was brought in to reconcile the ratings.

Functional quality control included thresholds for the metrics of framewise displacement (FD) and DVARS (D referring to temporal derivative of timecourses, VARS referring to RMS variance over voxels) as cutoffs for exclusion (FD ≤ 0.5mm, DVARS ≥ 50 change in % BOLD). Subjects with missing functional data were excluded also. The signal to noise ratio (SNR) was computed by averaging signal intensity and dividing it by standard deviation over time; as explained by Yeo and colleagues (2011), this was used as additional exclusion criteria, with images with an SNR of <100 over the whole brain being excluded from the analysis. After preprocessing was complete, 844 subjects remained in the dataset. When paired with available phenotypic and behavioral data, the analysis included 75 neurotypical subjects, 85 subjects with an ASD diagnosis, and 505 subjects with other varying diagnoses, including but not limited to anxiety disorders, depressive disorders, attention disorders, and communication disorders.

Parcellation and Functional Connectivity

Multi-session Hierarchical Bayesian Model (MS-HBM)

Brain parcellation is the division of the brain into functional networks that are similar in correlated activity at rest, as described in the introduction. Group level parcellations have identified common networks and their general locations, but there are idiosyncrasies in individuals that disappear at the group level (Braga & Buckner, 2017). This can be remedied by using individual parcellations rather than group-level parcellation to examine the networks we are most interested in.

Estimating individual parcellations of cerebral cortex can be done with multi-session fMRI data in the HBN dataset (Kong et al., 2019; Alexander et al., 2017). We binarized the functional connectivity profile for a particular vertex from a particular session and assumed that it is the same across all sessions. A more particular mathematical model is provided by Kong et al. (2019). In order to get the individual parcellation, the assumption must be made that each network has a distinct functional connectivity profile in a resting state scan. Variability can then be compared both across individual subjects (inter-subject variability) and across sessions of a single subject (intra-subject variability) (Kong et al., 2019; Yeo et al., 2011).

Generate Profiles and Initialization Parameters

This first step of the parcellation created connectivity profiles for each subject, and then averaged them across all subjects and all sessions to create a group parcellation of 17 resting-state networks (Figure 2). Cortical networks were defined as sets of regions with similar corticocortical functional connectivity. Following previous studies (Yeo et al., 2011; Fishl et al., 2004), the connectivity profile of a cortical region/vertex was defined as its functional coupling to 1,175 ROIs (Yeo et al., 2011). The ROIs consist of single vertices uniformly distributed across Freesurfer's fsaverage5 surface mesh. For subjects with only one functional run, the run was split into two sessions to create a uniform number of sessions across all subjects. If a subject had more than two functional runs, only two sessions were used.

A clustering approach using von Mises-Fisher distributions as described by Yeo et al. (2011) was used to identify the cortical networks, applied to estimate networks of cortical regions with similar profiles of connectivity (Lashkari et al., 2010). Previous stability analysis by Yeo et al. (2011) identified 7 to 17 distinct networks. We used the 17-network model as outlined by Kong et al. (2019).

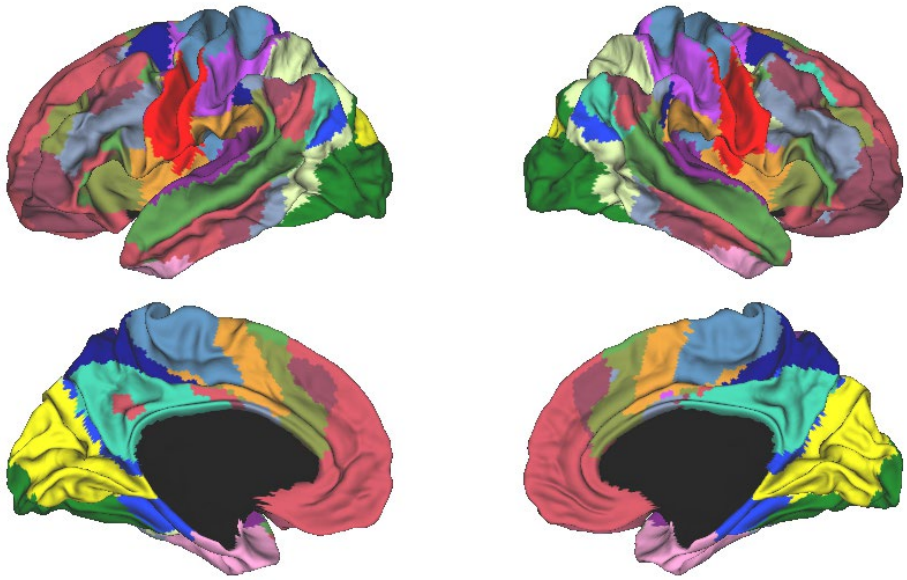

Figure 3: Group-level 17-network parcellation, mapped onto the cortical surface in fsaverage5 space.

Estimate Group Priors

This step of the parcellation method estimated the group priors in the MS-HBM. This prepares for the third step of the parcellation, where individual parcellations are generated for each subject. Priors estimated in this step include inter-subject functional connectivity variability, group-level connectivity profiles for each network, intra-subject functional connectivity variability, and a spatial prior which defines the probability of each network occurring at each location according to the ROIs (Kong et al., 2019).

Generate Individual Parcellations

Optimal parameters for parcellation were determined by calculating the homogeneity with several combinations of the w and c parameters. Then, we averaged the homogeneity values for all combinations of w and c in 30 validation subjects and found the highest homogeneity value. The w and c parameters with the highest average homogeneity in the validation set were used for all other datasets described in the current study.

Individual parcellations were generated using the same method as the group-level parcellation, with a slight alteration. Instead of averaging the connectivity profiles across all subjects, the algorithm used the connectivity profiles generated for each subject previously combined with the weight of the group spatial prior and the weight of the MRF smoothness prior generated previously to estimate the 17-network parcellation for only that subject (Kong et al., 2019). A Hungarian Matching algorithm was used to match the networks from our atlas to those described by Kong et al. (2019).

Quantitative Evaluation

According to Kong et al. (2019), one of the major obstacles we face in evaluating quality of individual parcellations from a resting state scan is the lack of ground truth. Previous studies have used a few different methods of quantitative evaluation, including resting-state connectional homogeneity and task functional inhomogeneity measures (Gordon et al., 2016; Chong et al., 2017; Gordon et al., 2017; Shaefer et al., 2017). These techniques assume that if an individual parcellation captures specific organization, then the networks specified should have homogeneous connectivity and function. As mentioned before, functional connectivity has been defined in this study as correlated activity between seed regions in the brain in a resting state. Resting-state connectivity was calculated by averaging correlations between resting-state fMRI time series of pairs of vertices within each identified network (Shaefer et al., 2017). These average values were then averaged across all networks, accounting for network size. The quantitative functional connectivity measures used in this analysis are the time series comparisons for every region of interest (Figure 3).

We extracted the surface area of each network for each subject and calculated network similarity using the Sørenson-Dice coefficient. Surface area was obtained from the individual

parcellation, and is defined by the area of the cortical surface corresponding to each network. The Dice coefficient was computed from the parcellations of each subject, and calculated as the overlap between corresponding networks of the test subject and the group parcellation.

These measures were chosen because of their ability to provide a representation of differences in brain topology, specifically pertaining to network organization. Since we are trying to understand how brain features relate to behavior, it is important to have a good picture of how those features differ between individuals, or our effects would disappear. Surface area of each network and the network similarity are two measures that help us get a clear picture. The functional connectivity measures then allow us to introduce the relationship of function to the measures of structure for a more complete understanding.

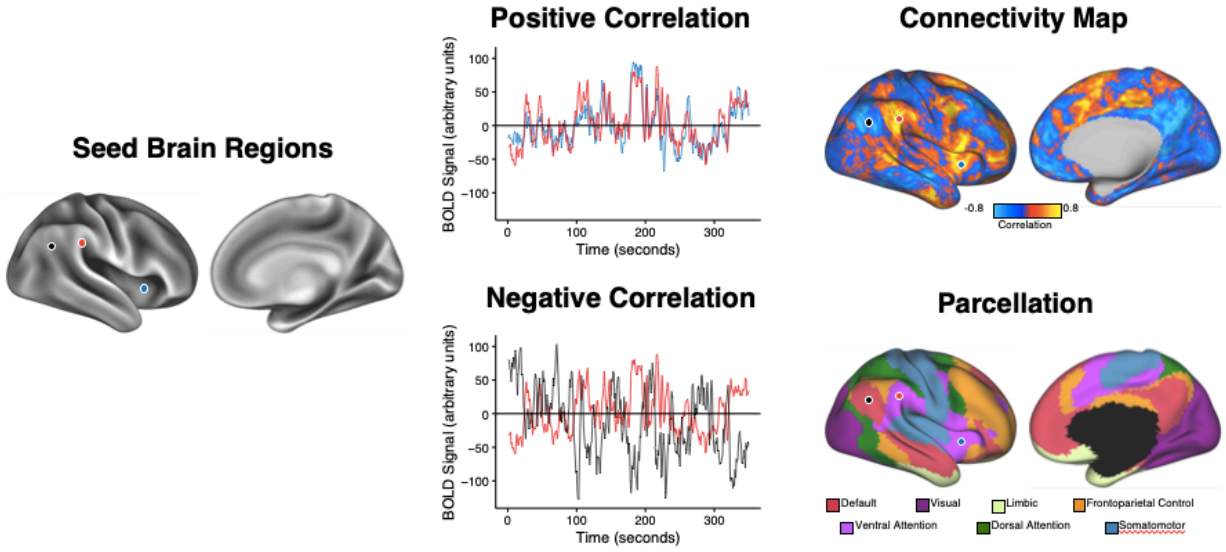

Figure 4: Visualization of functional connectivity measures as used in this study.Greater connectivity is defined as positive correlation in time series, or areas active at the same time. Lesser connectivity is defined as negative correlation or anti-correlation in time series, or areas active at different or opposite times.

Predicting Behavior from the Organization of Brain Networks

We used a kernel regression model as outlined by Kong et al. (2019) to predict behavioral phenotype in each individual as the weighted average of the behaviors of the training subjects in

the model. This model aims to estimate the conditional expectation of the response variable by characterizing a non-linear relationship between the explanatory variables, (i.e. surface area, network similarity, and functional connectivity), and response variables (i.e. performance on the behavioral tests). This allows for prediction of behavioral measures from functional connectivity profiles in networks and sub-networks across typical individuals and individuals with ASD.

Kernel Ridge Regression

The kernel ridge regression is a machine learning algorithm designed to predict the response variable based on the similarity of particular features of the test subject to the training subjects (Murphy 2012). This kind of analysis is advantageous for its flexibility, but presents a danger of overfitting the model. To reduce the likelihood of overfitting, an L2-regularization term was included in our analysis (Kong et al., 2019). We used a correlation kernel and 20-fold cross-validation to determine the regularization and kernel hyperparameters. Two folds were randomly separated out as the training validation sets to train and test the parameters, respectively, and establish hyperparameters. The cross validation was applied 20 times to the remaining or test fold with inner loop cross validation using the trained parameters. The L2-regularization parameter as well as the optimal hyperparameters are determined using the inner loop cross validation and then applied to predict the behavioral phenotype. To reduce the risk that the data may be sensitive to a particular splitting of the data (Varoquaux et al., 2017), 100 different splits were repeated, and the mean accuracy and standard deviation across all splits are reported (Kong et al., 2019). Prediction accuracy was measured by comparing the predicted and actual behavioral measures across all subjects within the test fold (Finn et al., 2015). This was repeated for each test fold in the 20-fold cross-validation, which output 20 accuracies that were then averaged to yield the accuracy for each split of the data (Kong et al., 2019).

Before running the kernel regression, age, sex, study site, body mass index, and total brain volume were regressed from the predictor variable, as these measures are known to correlate with motion (Siegel et al., 2017). Nuisance regression was also performed on training folds and the regression coefficients applied to the test folds during cross-validation (Kong et al., 2019).

Group Analyses

Group analyses were done to investigate whether there were any differences in functional connectivity, surface area, or network similarity between diagnostic groups. Analysis of Covariance (ANCOVA) was used to examine differences in groups while accounting for age, sex, study sight, total brain volume, and body mass index. False discovery rate (fdr) was used to correct for multiple comparisons, and Tukey HSD post-hoc tests were done for pairwise comparison significant connectivity features. In order to compare each pair of diagnostic groups a linear model was fit to each connectivity feature, and fdr was again used to correct for multiple comparisons.

Results

Behavioral Measures

Social Responsiveness Scale-2 (SRS)

The SRS measures social ability in children and is specifically tailored to identify social impairment in autistic individuals. When analyzing SRS scores across diagnostic groups using a one-way ANOVA, we found a group difference ($F = 108.7$, $p = 2e\text{-}16$). Post-hoc test of Tukey's HSD showed $p < 0.001$ for all pairwise comparisons. Figure 4 shows raincloud plots of the distribution of SRS scores by diagnostic group.

Social Communication Questionnaire (SCQ)

The SCQ is designed to evaluate social skill as well, but specifically communication skill in autistic individuals. A one-way ANOVA was also performed to compare score across diagnostic group, which yielded an initial F-statistic of 93.06 and a p-value of p = 2e-16, and Tukey's HSD showed $p < 0.001$ for all pairwise comparisons. Figure 5 also shows raincloud plots of the distribution of SCQ scores by diagnostic group, highlighting this correlation.

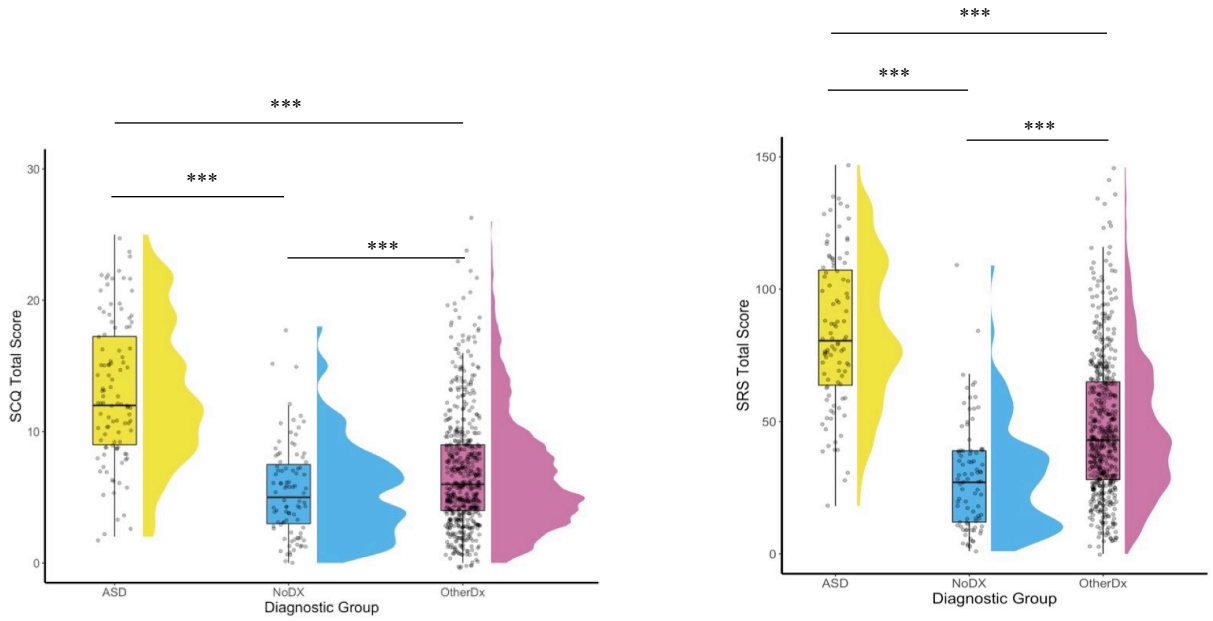

*Figure 5:Raincloud plots of SCQ and SRS score by diagnostic group. SCQ F = 93.06, p = 2.0e-16, SRS F = 108.7, p = 2.0e-16. Tukey's HSD for all pairwise comparisons yielded p < 0.001 for both tests. (*** = p < 0.001).*

Predicting Social Function Using Brain Features

Prediction of Social Function Using Global Brain Features

Initially, we implemented a kernel ridge regression model that included all the subjects from all the diagnostic categories and all the brain features (i.e. all pairwise functional connectivity features, the networks surface area features, and the network similarity features). The brain features were unable to predict either measure of social ability (Figure 6). The average

correlation between the brain features and the SRS scores was r = 0.00197, p > 0.1, and between the brain features and the SCQ scores was r = -0.063, p > 0.1.

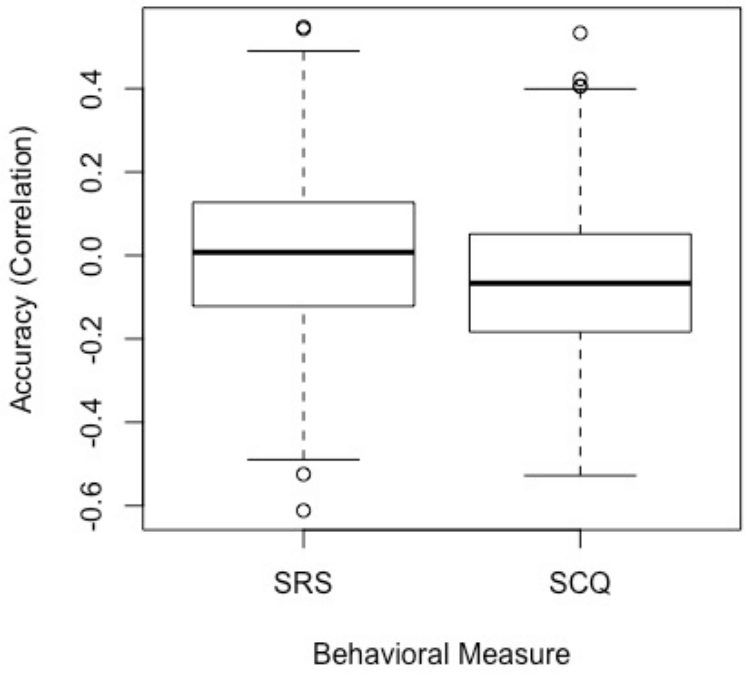

Figure 6:Prediction accuracy by behavioral measure. Correlation coefficients were averaged across 20 inner folds of cross validation then across 100 splits of the data. SRS r = 0.00197, p > 0.1, SCQ r = - 0.063, p > 0.1.

When we examined prediction accuracy of social ability using all the brain features but only including subjects within a specific diagnostic group, the results were similar with average correlation coefficients that were not predictive of performance on behavioral tests (Figure 7) (SRS: ASD r = 0.0837, p > 0.1, Neurotypical r = 0.1240, p > 0.05, Other diagnosis r = 0.0543, p > 0.1; SCQ: ASD r =-0.1171, p > 0.1, Neurotypical r = 0.0387, p > 0.1, Other diagnosis r = -0.0155, p > 0.1). A table of critical values was used to obtain p-values for the significance of the correlation coefficients.

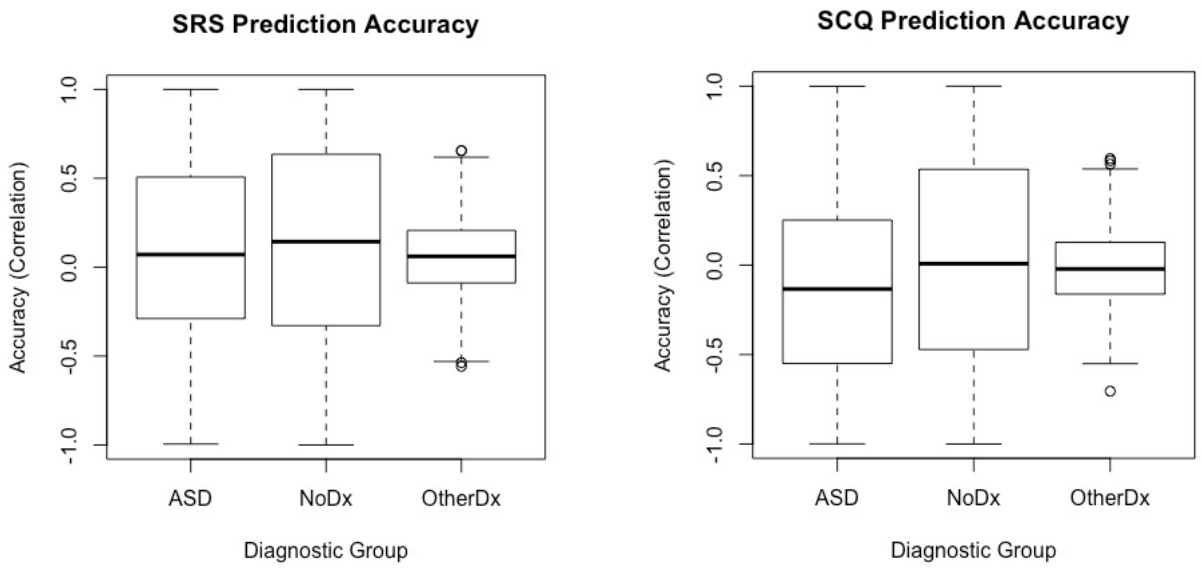

Figure 7: Prediction accuracy of SRS and SCQ behavioral measures by diagnostic group (SRS: ASD r = 0.0837, p > 0.1, NoDx r =0.1240, p > 0.1, OtherDx = 0.0543, p > 0.1; SCQ: ASD r = - 0.1171, p > 0.1, NoDx r = 0.0387, p > 0.1, OtherDx r = - 0.0155, p > 0.1).

Network-Specific Prediction of Behavior

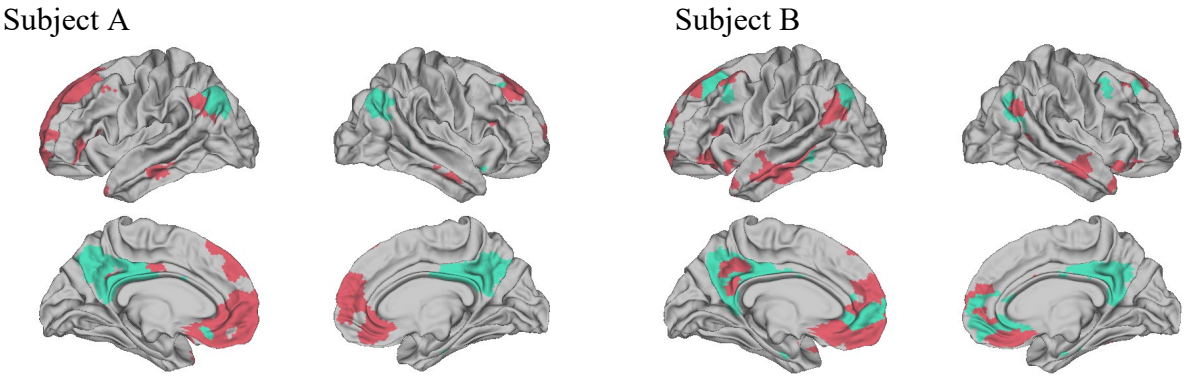

Figure 8:Visualization of two individual parcellations of DN A and DN B projected onto the cerebral cortex in fsaverage5 space.

To test whether DN A or DN B features specifically were able to predict behavior, after global analyses, we extracted the features relating specifically to these networks. We performed a kernel ridge regression model on all subjects in all diagnostic groups to examine the predictive ability of the functional connectivity, surface area, and network similarity features, but found very similar results to the global analysis (Figure 9) (DN A: SRS r = 0.0167, p > 0.1, SCQ r = - 0.0843, p > 0.1; DN B: SRS r = 0.0852, p > 0.1, SCQ r = 0.02752, p > 0.1).

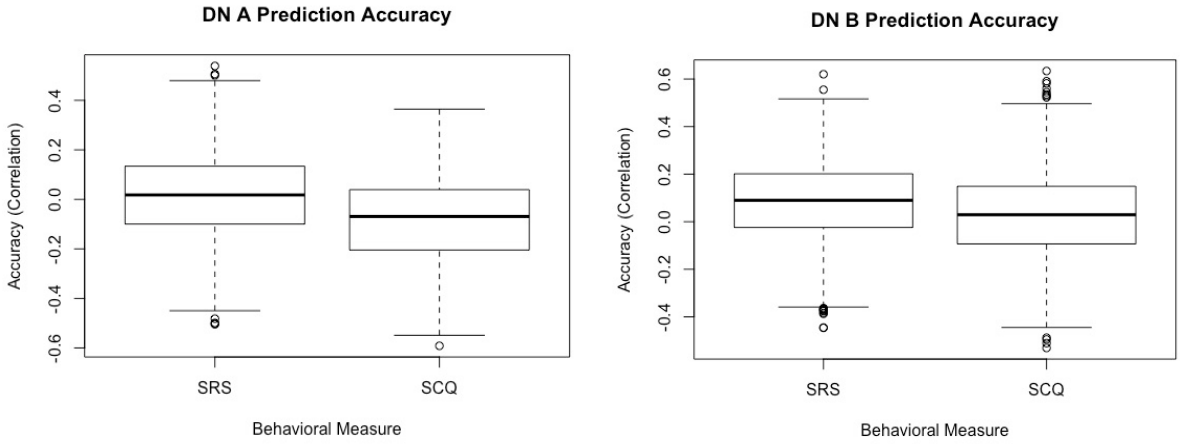

Figure 9: Prediction accuracy of behavioral test score for DN A and DN B. For DN A, SRS r = 0.0167, p > 0.1, SCQ r = - 0.0843, p > 0.1. For DN B, SRS r = 0.0852, p > 0.1, SCQ r = 0.02752, p > 0.1.

Looking at the results by diagnostic group, we again see no predictive ability of the functional connectivity features, surface area, or network similarity on the behavioral measures we examined for DN A (ASD: SRS r = 0.0179, p > 0.1, SCQ r = - 0.0958, p > 0.1; Neurotypical: SRS r = - 0.00127, p > 0.1, SCQ r = 0.0197, p > 0.1; Other Diagnoses: SRS r = 0.0944, p > 0.1, SCQ r = 0.0779, p > 0.1). However, for DN B, we find significant prediction accuracy for the behavioral measures in the autism group, with the prediction specifically of SCQ test score being significant (ASD SRS r = 0.213 p < 0.1, SCQ r = - 0.3654, p < 0.01; Neurotypical SRS r = - 0.0417, p > 0.1, SCQ r = 0.1388, p > 0.1; Other Diagnoses SRS r = 0.0891, p > 0.1, SCQ r = 0.0852, p > 0.1). We imposed $\alpha = 0.1$ based on other studies evaluating similar measures, feeling that this is a reasonable cutoff for the severity of the statistical methods we were using (Kong et al., 2019). The other diagnostic groups had very similar prediction accuracies to what we had previously seen (Figure 10).

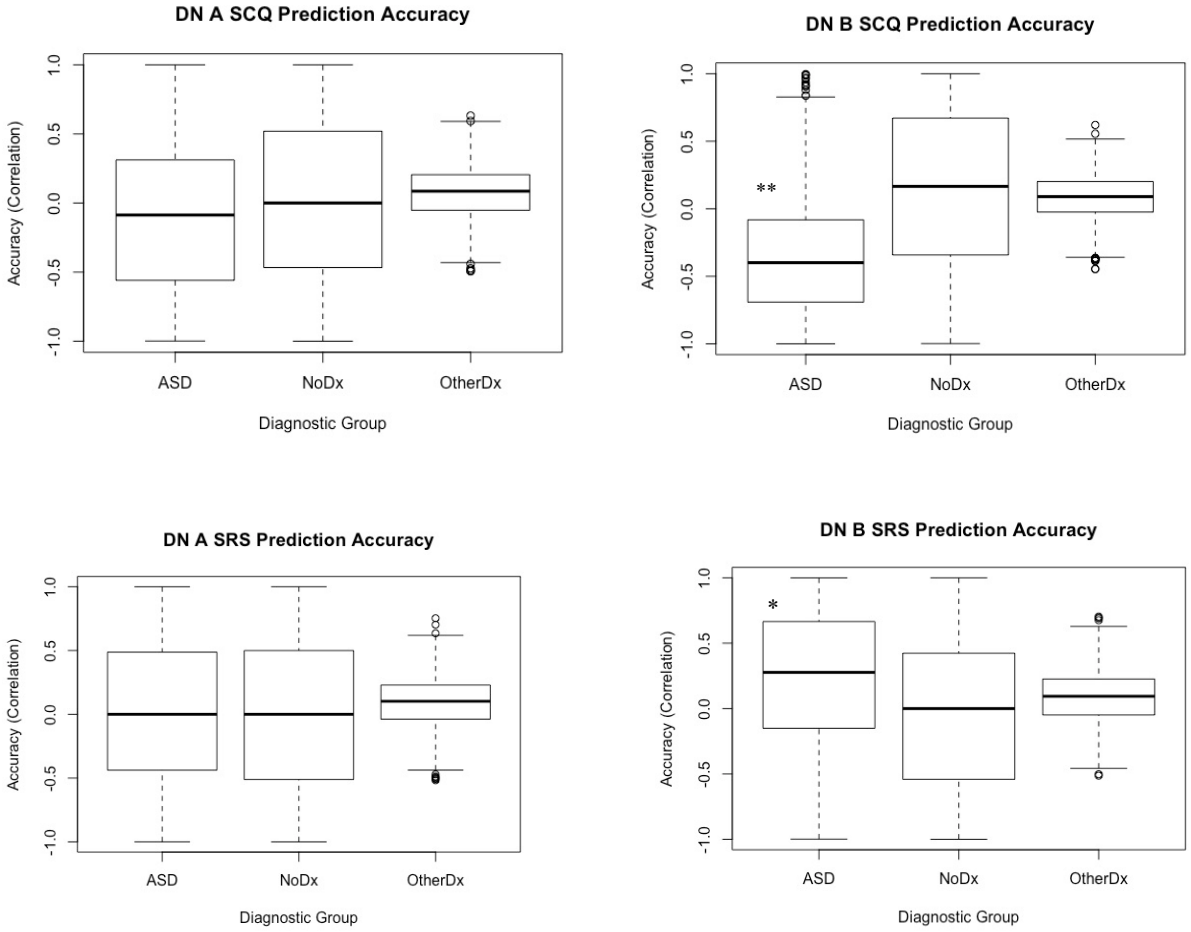

Figure 10: Boxplots of average correlation coefficients for each behavioral test by group for the individual networks DN A and DN B.

As seen above, the correlation coefficients are much closer to 0 for DN A, and for DN B closer to ~0.2 for the autism group. The other diagnosis and neurotypical groups, however, had correlation coefficients remaining close to 0.

Univariate Group Analysis

Surface Area and Network Similarity

Analysis of the network surface area of the networks of interest, DN A and DN B, was done using ANCOVA. When assessing surface area of DN A, accounting for age, sex, study site, BMI, and total brain volume, there was no statistically significant difference in surface area

between any group (F = 0.637, p = 0.529). This was likewise true for surface area of DN B (F = 1.896, p = 0.151). Thus, we can conclude that from this test, there is no difference in DN A or DN B network surface area across the diagnostic groups (Figure 11).

Another ANCOVA was performed to assess network similarity or the variation of the average dice coefficient across diagnostic group. Similar to network surface area, no significant difference was seen in the initial ANCOVA (F = 1.096, p = 0.335). Thus we can also conclude that there is no difference in DN A or DN B network similarity across the diagnostic groups when it comes to network similarity to the group (Figure 12).

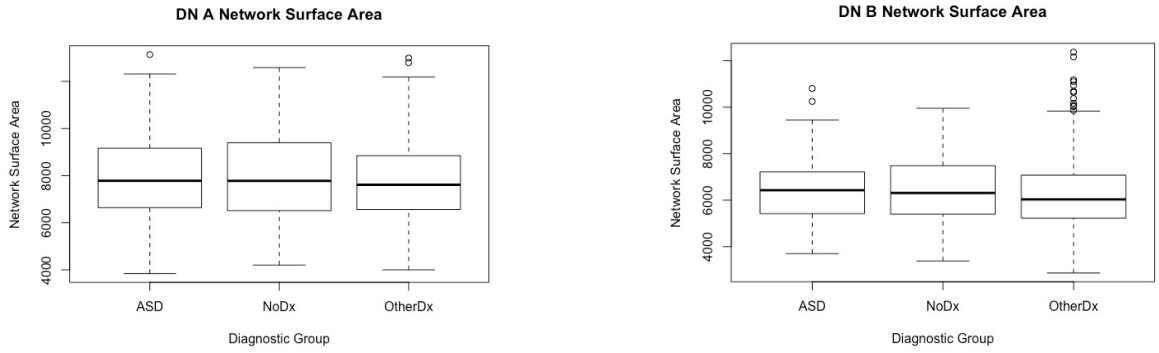

Figure 11: Boxplots of network surface area across diagnostic groups for DN A and DN B. For DN A, f = 0.637, p = 0.529, and for DN B, f =1.896, p = 0.151.

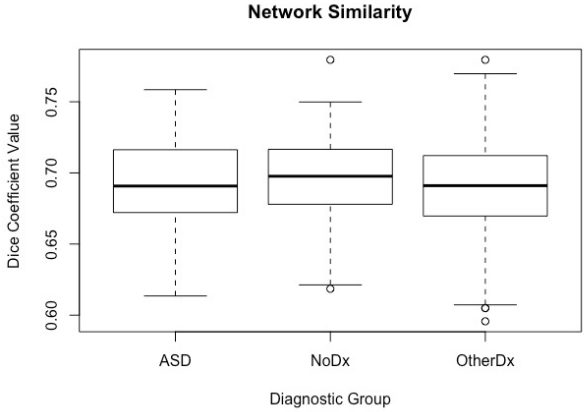

Figure 12: Boxplot of the Sørenson Dice Coefficient representing network similarity across diagnostic group. f = 1.096, p = 0.335.

Functional Connectivity

Analysis by group of all functional connectivity features was done using ANCOVA, correcting for age, sex, study site, BMI, and total brain volume. Correction for multiple comparisons was done using fdr. After correction for multiple comparisons, no functional connectivity features were significantly different based on diagnostic group (Figure 13).

Figure 13: Histogram of p-values for all features before (left) and after (right) adjustment by FDR correction for multiple comparisons. After correction for multiple comparisons, no p-values remained under p = 0.2.

Further analysis was done by comparing each pair of groups using a linear model, accounting for age, sex, study site, BMI, and total brain volume. No significant difference was found between any of the groups for any of the functional connectivity features (Figure 14).

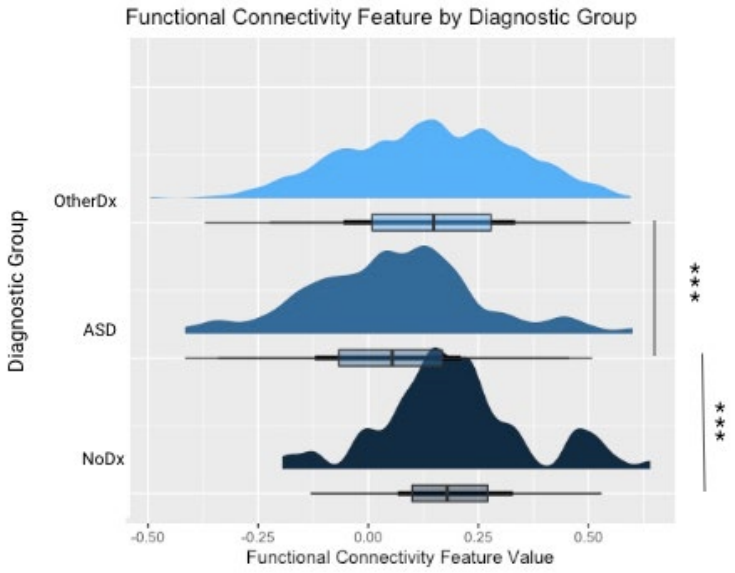

Figure 14: Raincloud plot showing the value of the functional connectivity feature (the comparison between one vertex and all others) by diagnostic group.

The same procedure was done using the features only from our networks of interest, DN A and DN B. ANCOVA analysis was done first across all subjects in all diagnostic groups, correcting for age, sex, study site, BMI, and total brain volume. Correction for multiple comparisons was done using fdr. This analysis yielded no significant differences between groups for DN A (Figure 15), and one significant value between diagnostic groups in DN B (Figure 16) ($F = 11.19$, $p = 0.0082$). This value corresponded to the functional connectivity timeseries comparison between the seed region 1 in the left lateral prefrontal cortex and the seed region 2 in the right ventral prefrontal cortex (Figure 17). Tukey HSD pairwise comparison showed differences between the autistic group and both others, but not between the neurotypical and other diagnoses groups (ASD vs NoDx $p = 0.0000147$, ASD vs OtherDx $p = 0.000547$, NoDx vs OtherDx $p = 0.0516$).

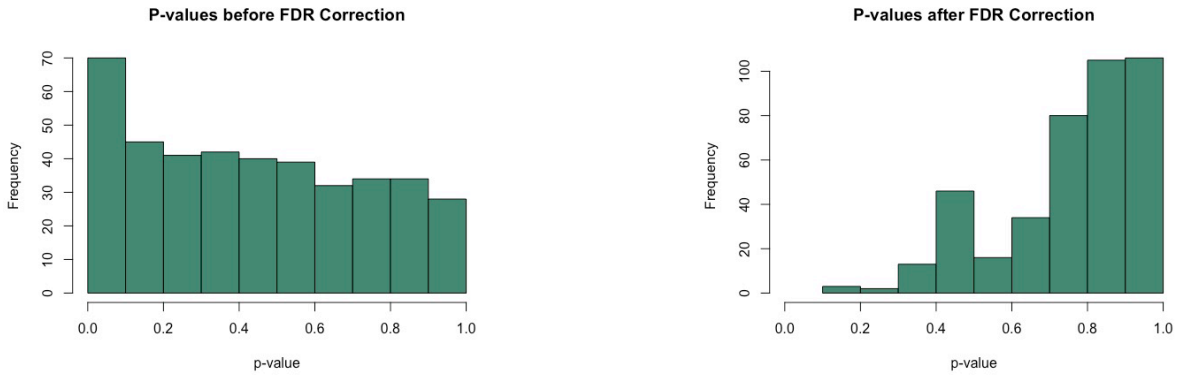

Figure 15: Histogram of p-values for functional connectivity features in DN A before (left) and after (right) fdr correction. After correction for multiple comparisons, no p-values were less than p = 0.1.

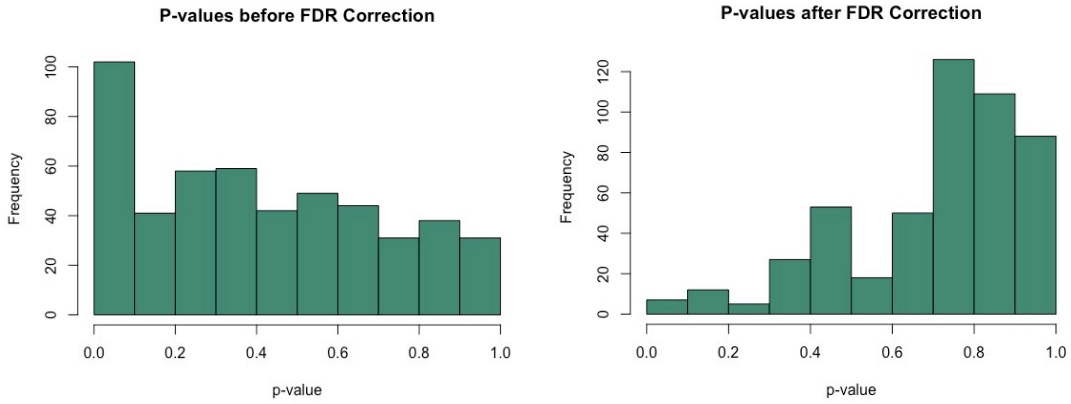

Figure 16: Histogram of p-values for functional connectivity features in DN B before (left) and after (right) fdr correction. After correction for multiple comparisons, one p-value was significant (p = 0.0082), corresponding to the comparison between seed region 1 in the left lateral prefrontal cortex and seed region 2 in the right ventral prefrontal cortex in DN B. All other p-values were greater than p = 0.05.

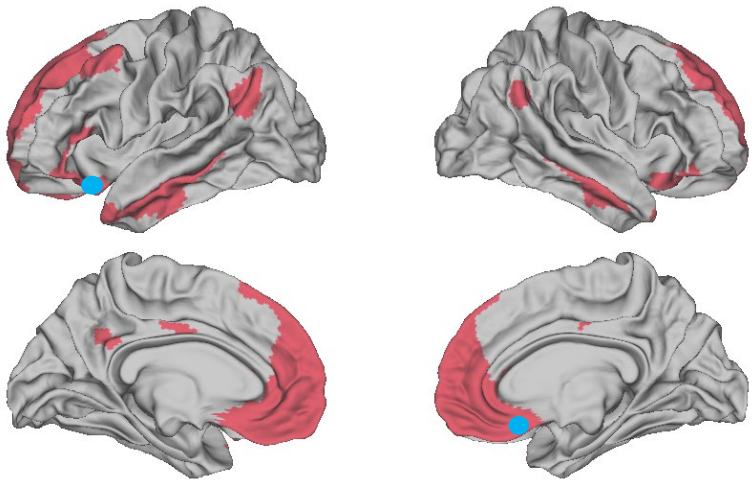

Figure 17: Group parcellation of DN B projected to the cortical surface in fsaverage5 space. Two blue dots represent regions of interest corresponding to the significant functional connectivity feature. P = 0.0082 after fdr correction, and Tukey HSD showed that the ASD group differed from the other two groups (ASD vs NoDx p = 0.0000147, ASD vs OtherDx p = 0.000547. No difference was found between the NoDx and OtherDx groups.

Discussion

These analyses showed no predictive ability for global brain features of performance on SRS or SCQ tests. We also showed no difference in global functional connectivity, surface area, and network similarity across diagnostic group. When we examined DN A and DN B specifically, we found that DN A features are not able to predict SRS or SCQ score in any group, but that DN B was able to predict scores in the ASD group. We found no differences across diagnostic group for functional connectivity features in DN A and one significantly reduced functional connectivity feature in DN B in the autistic group.

Individualized Parcellation and Connectivity

As discussed in the introduction, examining network connectivity at an individual level is essential to understanding the idiosyncrasies of the brain. These idiosyncrasies allow us to define networks more precisely as when we average across multiple brains, we lose clarity. Using individual analyses more clearly helps us define the effects we see, and is a more accurate way of assessing effects across the brain. Looking at the network parcellation of the group versus individuals, it is clear there are idiosyncrasies that could affect analyses if averaged into the group parcellation (Figure 18).

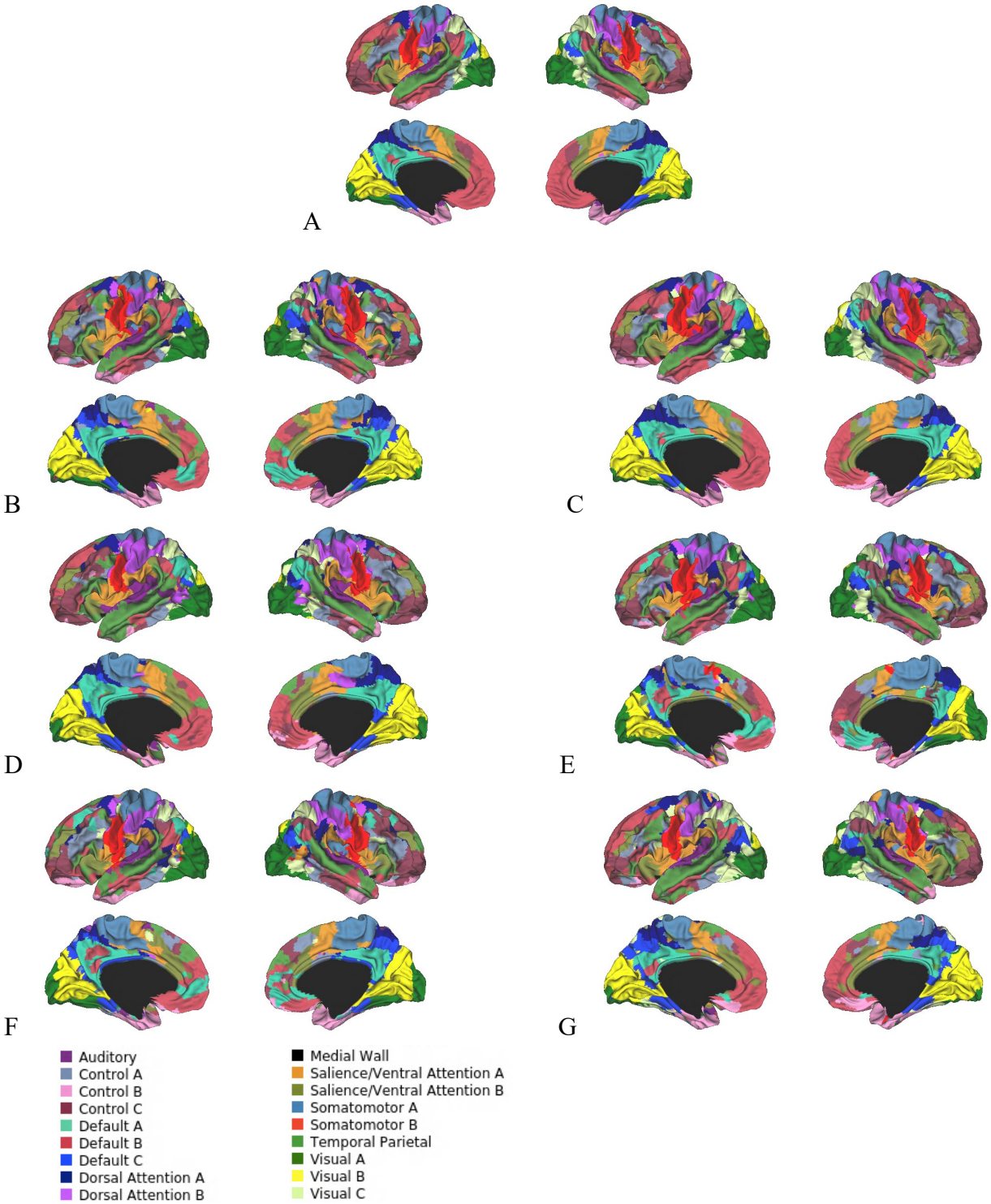

Figure 18:17-network parcellations of 6 individual subjects (B-G) projected onto cortical surface in fsaverage5 space, compared to group parcellation (A).

Prediction Accuracy and Significant Connections

Correlation Differences in Networks

When using the multivariate predictive model, for all features and all networks, we saw no correlation, and for DN A features we saw no correlation. For DN B, however, we saw a significantly higher correlation, specifically for the ASD group. This suggests that in the ASD group, functional connectivity features can predict behavioral performance on the SRS or SCQ behavioral assessments. The prediction accuracies for both behavioral tests are greater than many other correlations yet found for prediction of behavior. The original developer of this method was able to reliably predict behavior with correlation coefficients of approximately 0.13 (Kong et al., 2019). This is interesting as well because there was no real difference in the predictive ability of DN B features for the neurotypical or other diagnosis groups, the results matched with what we had seen previously in global and DN A analysis.

There are several reasons why we believe we may have observed this difference in predictive ability between diagnostic groups. First, as explained in the introduction, we know that autistic behavior is characterized by social difficulties. We also have explained that score on these behavioral measures is heavily dependent on diagnosis. It could simply be that there is no evidence we can pinpoint in the brain that will predict social performance for typical individuals. For the other diagnosis group, it is likely that there is some washing out of any effect because there are so many diagnostic groups lumped together. It could potentially be that there are effects that may be observed if we looked at other diagnostic groups individually, such as anxiety disorders or depressive disorders, but the purpose of this study was to examine an autism sample.

Significant Connectivity Features

In our analysis, we also discovered one feature in DN B that was significantly different between diagnostic groups. This feature corresponded to the comparison of time series of seed region 1 in the left lateral prefrontal cortex and seed region 2 in the right ventral prefrontal cortex, and was significantly different between the ASD and other two groups. This significant difference didn't show up in our global analysis, only in our network-specific analysis. This finding again highlights the importance of very specific analysis designs, as effects can be easily eclipsed.

Implications

Implications of null findings are sometimes difficult to infer, as conclusions can be drawn only from what is seen in the analysis. We have concluded based on our analysis that using this model with this data, we cannot globally define differences in functional connectivity, brain surface area, or network similarity based on diagnostic group, nor can we predict behavior on the selected behavioral tests from the features we examined when we examine all together. However, this does not necessarily mean that there is no potential for doing so; increasing sample size and examining different behavioral measures may yet yield different results (Marek et al., 2022).

Examining DN B, however, did yield some results that highlight potential differences in an autistic population versus a population of neurotypical individuals or individuals with other diagnoses. These findings potentially point to differences that may be observed with more research focused in the right areas. This research may have to be done using different techniques, as the differences are likely very subtle, as up to this point we have not been able to find reproducible differences in brain topology in individuals with autism.

In a broader perspective, this study adds to the body of research supporting the conclusion that it is very difficult to define autism based on biological or functional features in the brain. There are behaviors that can be clearly defined as common to autistic people, but in this as in some other cases, there is no support to suggest those behavioral differences can be isolated at a global level. On a network level, this study shows that there is potential for identifying differences, but these results must be reproduced in order to use them to classify biological characteristics of autism. As described in the introduction, there is some evidence to support behavioral differences at both global and network level, and conflicting findings continue to abound in this area of study. Marek et al. (2022) argue that because many studies have a tendency to be biased in favor of significant and large effects, this tendency limits publication of null results. Many of these results however are inaccurate because of inflated effect sizes that come along with small sample sizes.

The Bigger Picture

There is a large body of autism research that points to similar conclusions as this study, as well as research that points to differing conclusions. To highlight a few, this study found no difference across diagnostic groups in functional connectivity features, meaning that we cannot say that there is either hypo- or hyperconnectivity in the autistic group compared to the others. However, quite a few studies have found either hypoconnectivity (Bernas et al., 2018; Moseley et al., 2015; Murdaugh et al., 2012; Zeng et al., 2017; Tang et al., 2020) or hyperconnectivity when comparing global connectivity in an autism sample to a neurotypical sample (DiMartino et al., 2014, Cerliani et al., 2015; Keown et al., 2017; Jann et al., 2015).

This study also found no difference across diagnostic group in DN A, and one different feature in DN B. Previous studies of the DN in autism have shown altered connectivity in the DN

to be associated with autistic symptoms (Jann et al., 2015, Lynch et al., 2013, Maximo et al., 2013, Pereira et al., 2018). This study showed that connectivity features in DN B in an autism sample are predictive of social cognition and support these studies that say connectivity is correlated with symptoms. However, this study does not show that there is any difference in connectivity between groups, as mentioned above.

To date, other autism studies have not focused on the sub-networks of the DN, and so this study provides new evidence showing that DN B is more involved in ASD symptomology. Further research is needed to further illuminate that role and understand how DN B functioning might affect autistic symptoms and how it may be a source of the biological mechanisms of autism.

Limitations

Although one of the goals of this study was to use a larger sample size to understand the differences in functional connectivity, it did still end up being one of the limitations; after processing the data and pairing it with available phenotypic data, we still had relatively few subjects with an autism diagnosis and relatively few neurotypical subjects from this dataset. According to Marek et al. (2022), in order for brain-wide associations to be reproducible, we need a sample size of thousands of individuals. This study still had a sample size of about 3 times the median (75 subjects as opposed to about 25), it is still far from where it needs to be to be truly reproducible. We know that the bigger the sample size, the more reliable the results are, and the more robust the effects. It may be that this analysis was simply too small to show reliable or major differences in functional connectivity, or to reliably predict performance on behavioral tasks associated with autism. Further study is required to determine if these results, specifically those that we found in DN B, are robust and continue with a larger sample.

Another improvement that could be made in future studies is the increase in scanning time. This dataset provided a relatively consistent amount (~23 mins) for each participant, but others have shown that increasing scanning time has only positive effects on robustness and consistency of analysis (Nielsen et al., 2013, Marek et al., 2022).

In this study, data collection site was regressed out of the predictor variable in our analyses, but in this particular dataset collection parameters varied slightly between sites. There was not a large amount of variation, but more care might be taken in the future to understand how these slight differences can affect data processing and outcome of analysis.

Another point made by Marek et al. (2022) is that univariate analyses in particular are very vulnerable to small effect sizes, and require very large sample sizes in order to have applicable and reproducible results. The kernel ridge regression predictive model that we used to obtain correlation was a multivariate model and more robust, but other analyses were univariate and so vulnerable to this kind of reproducibility and small effect issue. Developing another multivariate method in order to assess differences between groups would be a wise next step to understand if the effects we have seen are robust.

Conclusion

These analyses have shown that first, compared to other groups, the autistic sample did not differ in features of functional connectivity, network surface area, or network similarity. Second, DN B, which is involved in social cognition, was able to predict behavioral performance on social measures for the autism group. These results add to the body of evidence in autism research showing that there are some discernible effects in autistic samples compared to other groups that are reproducible when examined at an individual level. More research needs to be focused on the default network and its sub-networks in order to more fully understand their role

in autism, but there is also potential in examining other network-specific traits to elucidate the role of specific brain areas in autism. Research to this effect will continue to help us understand better the biological mechanisms of autism.

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