NEURAL CORRELATES OF REPETITIVE BEHAVIOR IN AUTISM SPECTRUM DISORDER

A Thesis
Presented to the
Faculty of
San Diego State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Psychology

by
Angela Abbott
Fall 2013
SAN DIEGO STATE UNIVERSITY

The Undersigned Faculty Committee Approves the

Thesis of Angela Abbott:

Neural Correlates of Repetitive Behavior in Autism Spectrum Disorder

Ralph Axel Mueller, Chair
Department of Psychology

Pamela Moses
Department of Psychology

Bonnie Kraemer
Department of Special Education

Alan Lincoln
California School of Professional Psychology, Alliant University

8/13/13 Approval Date
ABSTRACT OF THE THESIS

Neural Correlates of Repetitive Behavior in Autism Spectrum Disorder

by

Angela Abbott

Master of Arts in Psychology
San Diego State University, 2013

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder associated with repetitive behavior (RB), which may impair daily functioning at a lower-order sensorimotor level (e.g. rocking back and forth) and a higher-order cognitive level (e.g. adherence to a routine). Evidence for brain abnormalities involved in RB is shown in volumetric studies indicating basal ganglia enlargement and functional studies indicating altered corticostriatal circuitry. Connectivity differences in the default mode network (DMN), involved in self-referential, baseline ‘resting-state,’ and the salience network (SN), involved in inhibitory functioning and directing attentional resources, may also have a role in RB symptoms. To investigate patterns of connectivity underlying RB in ASD, the current study examined two regions of interest (ROIs) in the striatum (dorsal caudate nucleus, dorsal caudal putamen), two in the SN (anterior insula, dorsal anterior cingulate cortex), and two in the DMN (ventral medial prefrontal and posterior cingulate cortex). Archival resting state functional magnetic resonance imaging (fMRI) data and repetitive behavior scores were obtained for overall 66 participants (35 ASD, 31 typically developing [TD]). Imaging data were motion-corrected and preprocessed using spatial smoothing, band-pass filtering and removal of nuisance regressors, including 6 rigid-body motion parameters and their derivatives, white matter and ventricular signals and their derivatives, and physiological time series. Average blood oxygen level dependent (BOLD) time series were extracted for each region of interest in each participant and correlated with all other voxels in the brain in a whole-brain analysis. The resulting Pearson’s correlation values were standardized using Fisher’s r-to-z transformation. One- and two-sample t-tests were then used to examine functional connectivity within and between groups. Results showed similar patterns of within-group functional connectivity; however, in between-group comparisons, mixed findings emerged with the ASD group showing mixed patterns of both over- and underconnectivity for each seed, except for the SN and dorsal caudate nucleus, for which only underconnectivity was detected. Clusters showing significant group differences in connectivity (mean z’ converted from BOLD signal correlations) were then correlated with cumulative total RB scores, using the Repetitive Behavior Scale–Revised along with separate cognitive and motor RB subscales, and several areas in which underconnectivity was associated with higher RB scores were found. Finally, network connectivity was assessed by calculating the correlation between time series for each ROI pair in each participant, separately for each group. Only the putamen and caudate nucleus ROI pair emerged as significant, with overconnectivity in the ASD group. Analyses conducted within the ASD group showed increased connectivity of the putamen, primarily with the DMN, correlating
with fewer RB symptoms. Furthermore, increased connectivity between the SN and DMN was associated with decreased symptom severity on the cognitive subscore of the Social Responsiveness Scale, a measure highly correlated with the RBS–R. Overall, results suggest that functional connectivity of the DMN, putamen, and – to a lesser extent – the salience network are associated with symptom severity and repetitive behaviors in ASD. Patterns of connectivity were largely decreased within networks and increased between networks, emphasizing the need for further examination of the segregation and interaction of separate functional networks in relation to characteristics of ASD.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>PAGE</th>
<th>CHAPTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>iv</td>
<td>1 INTRODUCTION .................................................................1</td>
</tr>
<tr>
<td></td>
<td>Repetitive Behavior In ASD ..................................................1</td>
</tr>
<tr>
<td></td>
<td>Neural Abnormalities and RB ................................................2</td>
</tr>
<tr>
<td></td>
<td>Functional Connectivity ......................................................3</td>
</tr>
<tr>
<td></td>
<td>Corticostrital Circuitry .....................................................4</td>
</tr>
<tr>
<td></td>
<td>Neural Networks and RB ......................................................6</td>
</tr>
<tr>
<td></td>
<td>Objectives and Hypotheses ..................................................8</td>
</tr>
<tr>
<td></td>
<td>2 METHODS .............................................................................9</td>
</tr>
<tr>
<td></td>
<td>Participants ...........................................................................9</td>
</tr>
<tr>
<td></td>
<td>Scales and Instruments ........................................................9</td>
</tr>
<tr>
<td></td>
<td>MRI Data Acquisition ..........................................................11</td>
</tr>
<tr>
<td></td>
<td>fMRI Data Preprocessing ........................................................11</td>
</tr>
<tr>
<td></td>
<td>Regions of Interest ..............................................................12</td>
</tr>
<tr>
<td></td>
<td>FcMRI Data Analysis .............................................................13</td>
</tr>
<tr>
<td></td>
<td>3 RESULTS ..............................................................................15</td>
</tr>
<tr>
<td></td>
<td>RBS–R Correlation with severity and demographics .......................17</td>
</tr>
<tr>
<td></td>
<td>FC Correlates of RB .............................................................18</td>
</tr>
<tr>
<td></td>
<td>Preprocessing Effects ...........................................................22</td>
</tr>
<tr>
<td></td>
<td>4 DISCUSSION ..........................................................................25</td>
</tr>
<tr>
<td></td>
<td>Within Network .......................................................................25</td>
</tr>
<tr>
<td></td>
<td>Between Network ......................................................................27</td>
</tr>
<tr>
<td></td>
<td>Outside Network ......................................................................28</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. Participant Characteristics .................................................................10
Table 2. Talairach Coordinates of Regions of Interest ........................................13
Table 3. RBS–R Correlations with Diagnostic and Behavioral Measures ..............19
Table 4 Associations Between RBS–R scores and Clusters Showing Group
  Connectivity Differences ..............................................................................22
LIST OF FIGURES

PAGE

Figure 1. Significant clusters of functional connectivity effects between TD and ASD groups for: (A) Salience network (SN). .................................................................16

Figure 2. Relationship between RBS–R Cognitive subscale and connectivity (z’) for: (A) dcP x DMN (r(18)=-.48, p=.009), (B) dcP x mPFC (r(18)=-.46, p=.013), and (C) dcP x PCC (r(18)= -.39, p=.043). ......................................................20

Figure 3. Relationship between SN x DMN connectivity (z’) and SRS Cognition subscales within ASD (r(33)=-.567, p<.001). ...............................................................21
I would like to thank the faculty and staff of the psychology department of San Diego State University for making possible the educational benefits of the master’s program in psychology. I am particularly grateful to my mentor, Axel Müller, for providing me with guidance throughout my thesis and with research training that will be useful in future endeavors. Thank you to BDIL lab members, past and present, who were always available to answer questions and were an invaluable resource for helping me learn data analysis techniques. I would also like to express my appreciation to my committee members for their contributions: to Pamela Moses for helping me conceptually refine the ideas presented, to Bonnie Kraemer for helping me ground the research from an applied perspective, and to Alan Lincoln for offering his clinical expertise and facilitating participant recruitment. My deep respect and special thanks also goes out to the participants and their families for giving their time and making research projects like this possible. Finally, thank you to my family and friends for their support and, especially, to my mother, to whom I will always be grateful for instilling in me enjoyment for learning and growth.
CHAPTER 1

INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder steadily increasing in prevalence (Centers for Disease Control and Prevention [CDC], 2012). Its core characteristics include qualitative impairments in social interaction and communication, and repetitive behavior (RB). ASD symptoms appear within the first three years of life and are accompanied by widespread brain abnormalities. RBs emerge early on in development and can have detrimental effects on learning, socialization, parenting, and family cohesion, all of which may negatively impact subsequent development (Boyd, McBee, Holtzclaw, Baranek, & Bodfish, 2009).

REPETITIVE BEHAVIOR IN ASD

RBs encompass a broad spectrum of behavioral manifestations, which vary in part depending on the severity of the disorder, but can be loosely categorized as “lower-order” or “higher-order” behaviors (Turner, 1999). Lower-order RB occurs in all babies as gross motor skills develop, but becomes symptomatic of ASD when it persists beyond the first year of life (Loh et al., 2007). Included in this category are stereotyped behavior, such as arm flapping, and self-injurious behavior, most commonly head-banging. Around two to three years of age, RBs take on a higher-order component characterized by restricted interests, adherence to a routine, and compulsions analogous to those seen in obsessive compulsive disorders (Arnott et al., 2010; Turner, 1999). Throughout development, RBs are linked with abnormal sensory processing (Gabriels et al., 2008) and have been shown to increase in visually stimulating environments (Colman, Frankel, Ritvo, & Freeman, 1976). However, rather than strictly impairing, RBs appear to serve a calming function in overstimulating environments and are associated with a reduced heart rate, particularly under distress (Willemsen-Swinkels, Buitelaar, Dekker, & van Engeland, 1998). While RBs correlate with anxiety (Rodgers, Glod, Connolly, & McConachie, 2012), one theory suggests that this helps
individuals with ASD compensate for an unstable arousal system by narrowing sensory input to a more restricted, controllable scope (Liss, Sauliner, Fein, & Kinsbourne, 2006).

**NEURAL ABNORMALITIES AND RB**

Studies of altered brain anatomy and function in ASD have provided some insight into the etiology of RB and its potential role in mitigating core symptoms. In anatomical studies, the basal ganglia have been closely scrutinized given this region’s known involvement in movement. Estes et al. (2011) found that decreased size of the putamen, but not the caudate, correlated with increased RB in pre-school age children, many of whom were low-functioning. In older children, caudate volume was shown to be increased in ASD relative to controls even after controlling for total brain volume. Interestingly, in two studies (Langen et al., 2009; Sears et al., 1999) increased caudate size was associated with decreased RB symptoms within ASD cohorts. This effect was moderated by age, with children and adolescents driving the correlation. In a sample of adults with ASD, however, opposite findings have emerged showing a positive correlation between caudate volume and RB (Hollander et al., 2005). In addition, altered growth trajectories in ASD are likely to play a role, as the volume of the head of the caudate nucleus has been found to increase with age in ASD, but to decrease with age in typical development (Langen et al., 2009). Taken together, findings suggest volumetric abnormalities in the striatum, some of which could potentially be related to compensatory changes.

Impaired cognitive control, commonly found in ASD, has been correlated with higher-order RB (Mosconi et al., 2009) and is consistently associated with altered activation of frontal areas. Cognitive control task paradigms, including target detection tasks with distracters and response set shifting tasks, have been shown to elicit hypoactivation of the rostral anterior cingulate cortex (rACC) (Dichter & Belger, 2008; Kana, Keller, Minshew, & Just, 2007) and the intraparietal sulcus in ASD, which in turn is associated with more severe RB (Shafritz, Dichter, Baranek, & Belger, 2008). A double case study involving SSRI treatment supported these findings showing a reduction in RB with concurrent increased rACC activation in one participant, and RB symptom worsening accompanied by increased activation in the supplementary motor cortex in another (Dichter et al., 2010). However, a hyperactive inhibition response has also been detected in the rACC (Thakkar et al., 2008) and
in the frontal eye fields, subserving the oculomotor network (Agam, Joseph, Barton, & Manoach, 2010; Alexander, DeLong, & Strick, 1986), both of which correlated with increased RB. Dichter, Felder, & Bodfish (2009) also detected overactivation during a shape detection task for the anterior cingulate cortex as well as the insula, important for basic sensory and interoceptive processing, and the frontopolar cortex, a region contributing to the highest levels of cognitive control (Badre, Hoffman, Cooney, & D'Esposito, 2009). The apparent discrepancy in fMRI activation findings in ASD may be due, in part, to sample variability of a heterogeneous population and differences in task paradigms, particularly in whether working memory is involved (Kana et al., 2007). Finally, increased activation in the supplementary motor area along with decreased activation in the cerebellum was found during a simple finger-tapping motor task (Mostofsky et al., 2009) suggesting altered recruitment of motor regions. Although mixed, these findings suggest that altered cognitive control and sensorimotor processing may both contribute to RB symptomatology. The investigation of functional brain networks may therefore lead to a better understanding of brain dysfunction underlying RBs in ASD than the focus on abnormalities at the level of single brain regions.

FUNCTIONAL CONNECTIVITY

Functional connectivity magnetic resonance imaging (fcMRI) has become the most commonly used technique for the study of functional brain networks in recent years. Similar to conventional activation fMRI, fcMRI is based on measuring changes in the blood oxygen level dependent (BOLD) signal. This signal reflects the hemodynamic response, in particular the replenishment of oxygen for firing neurons, and thus serves as an indirect indicator of neuronal activity (Huettel, Song, & McCarthy, 2009). Independent of stimuli-induced activation, spontaneous fluctuations occur in the BOLD signal at low frequencies and are thought to indicate endogenous neural properties and offer an unbiased measure of brain functioning. For particular brain regions, BOLD signals have a propensity to fluctuate in synchrony and may indicate processing for related tasks. Such functionally related but often spatially discrete areas, called functional networks, are thought to underlie mature brain dynamics (Fox & Raichle, 2007). Functional connectivity, therefore, is measured by examining the correlation in the BOLD signal between areas of the brain. While task-based
and ‘resting-state’ studies have yielded similar patterns of connectivity, the ‘resting-state’ may be especially useful in examining networks that predominate in the absence of a task, which have shown to be affected in ASD. Patterns of connectivity are best observed by isolating frequencies at which subtle, task-independent variations occur reflecting intrinsic connectivity properties (Van Dijk et al., 2010). This method of analysis may be particularly useful in gaining insight into the neurological profile of ASD as it is characterized by distributed dysfunction (Müller, 2007).

**Corticostriatal Circuitry**

Although long-distance underconnectivity is characteristic of the ASD brain (see Vissers, Cohen, & Geurts, 2012 for review), the striatum is one region for which functional overconnectivity has been reported (Di Martino et al., 2011; Turner, Frost, Linsenbardt, McIlroy, & Müller, 2006). A meta-analysis of 126 studies estimated the likely parcellation of the striatum based on differential connectivity patterns in neurotypical individuals (Postuma & Dagher, 2006). Results showed evidence of a rostral/caudal gradient in the putamen corresponding to prefrontal executive functioning connectivity rostrally and to primary and supplementary motor area connectivity caudally. In addition, a dorsal/ventral gradient in the caudate nucleus and putamen differentiated connectivity with associative and motor areas dorsally, including projections to the anterior cingulate cortex, insula, primary motor cortex, and premotor areas, and with limbic areas ventrally (Postuma & Dagher, 2006). In ASD, striatal seeds associated with functionally distinct networks have shown widespread dysfunction (Di Martino et al., 2011). The dorsal caudal putamen was connected in ASD, but not TD, with the parahippocampal gyrus, hippocampus, and right temporal-occipital fusiform gyrus, particularly for the right hemisphere. The ventral rostral putamen showed overconnectivity with the superior temporal gyrus and planum temporale, involved in language processing and social cognition (Di Martino et al., 2011). TD and ASD groups showed opposite connectivity patterns for the dorsal caudate, which showed positive connectivity in ASD but negative connectivity in TD for regions supporting sensory processing, namely the left temporal fusiform gyrus and bilateral supramarginal gyrus (Di Martino et al., 2011). Similarly, the ventral striatum showed positive connectivity with the left supramarginal gyrus in ASD but negative connectivity in TD. Additional
overconnectivity of the ventral striatum was seen with the anterior and mid-insula and the superior temporal gyrus, possibly reflecting impaired paralimbic processing and social reward deficits, respectively. Notably, all striatal seeds were overconnected to the pons, which is responsible for basic sensory and arousal processes. This finding could reflect impaired connectivity beginning early stages of development (Di Martino et al., 2011).

Trends in the data reported by (Di Martino et al., 2011) also suggested that RB may be related to connectivity between the dorsal caudal putamen/pons and the right ventral rostral putamen/superior temporal gyrus. The caudal putamen, which is thought to play a prominent role in initiating movement, could reasonably be assumed to impact RB; however, the RB measure used in this study was part of a diagnostic test and may not have been sensitive enough to the heterogeneous class of RB found in ASD to detect reliable neural associations. The superior temporal gyrus and ventral striatum are hypothesized to be involved in an anterior cingulate loop, one of five basal ganglia circuits described by Alexander et al. (1986), who posited that discrete parallel circuits connect the basal ganglia with the cortex. The anterior cingulate loop receives input from other areas showing abnormalities in ASD, such as the hippocampus and temporal regions (Alexander et al., 1986; Di Martino et al., 2011; Turner et al., 2006). Although the exact function of this loop remains speculative, all five proposed parallel corticostriatal circuits have been supported by fcMRI findings (Di Martino et al., 2008).

Another fcMRI study (Turner et al., 2006) reported diffuse overconnectivity of the caudate nucleus in the left hemisphere, which extended to the right middle frontal gyrus, left medial frontal gyrus, bilateral precentral gyrus, right postcentral gyrus, bilateral cingulate gyrus and the left cuneus. Altered connectivity with frontal areas as reported here may play a role in cognitive control deficits while diffuse connectivity of the caudate nucleus with sensorimotor areas may disrupt functioning of the motor loop which connects the putamen and motor and somatosensory cortices among other sensorimotor regions to form circuit (Alexander et al., 1986). This may reflect less differentiated striatal networks and provide a plausible link for cognitive control deficits and motor abnormalities.
Impaired connectivity in ASD has also been shown within sensorimotor and inhibition networks. For sensorimotor functioning, Mostofsky et al. (2009) reported decreased connectivity within a motor execution network, including the primary sensorimotor cortex, thalamus, cerebellum, and supplementary motor area, during a simple finger tapping task. Reduced connectivity with the somatosensory cortex has also been found for the posterior insula, which receives sensory information and connects to the motor thalamus (Ebisch et al., 2010). Likewise, the anterior insula, important in integrating interoceptive states with sensory input from the posterior insula, showed reduced connectivity with the amygdala and, in individuals with ASD but not Asperger syndrome, with the dorsal anterior cingulate cortex (Ebisch et al., 2010). This may reflect diminished emotional awareness of self and others and reduced ability to regulate emotions, respectively. Importantly, the anterior insula and dACC are key regions of the salience network, which is purportedly responsible for directing attentional resources and acting as a switch between the internally guided default mode network and the externally guided executive control network (Uddin & Menon, 2009). Interestingly, the dACC and the frontal eye fields, involved in an inhibitory network, showed increased functional connectivity in ASD, which correlated with increased RB (Agam et al., 2010). One view suggests that heightened inhibitory signals may be required in more severe cases of ASD, and consequently, may compensate for reduced functioning of the salience network. Such a dysregulated “switching” mechanism may explain reduced coherence within networks. In a separate study measuring structural covariance, correlations among regions of the salience network were reduced in ASD, specifically for the left insula and anterior cingulate as well as bilateral frontal regions and the anterior temporal cortex (Zielinski et al., 2012). The same study found greater covariance between the right insula and bilateral motor areas in the ASD group. This supports findings of dysregulation among the salience network, but may also link processing of this network with motor regions.

Among the most robust findings involving correlations with RB are those related to the default mode network (DMN), which is implicated in self-reflection and mind-wandering in the ‘resting state’. Activation studies have shown less DMN suppression during task performance in ASD relative to controls (Kennedy & Courchesne, 2008), especially under
high perceptual load conditions (Ohta et al., 2012). Altered processing may be the result of reduced coordination among regions of the network. Weng et al. (2010) found reduced connectivity of the left posterior cingulate cortex in 9 out of 11 DMN regions. Among these, connectivity to the medial prefrontal cortex, temporal lobes and superior frontal gyri was negatively correlated with RB in adolescents with ASD. In particular, the ventral medial prefrontal cortex (vmPFC) may be particularly affected by decreased network connectivity and has been shown to exhibit greater connectivity during self-referential compared to other-referential processing (Lombardo et al., 2010). Interestingly, in ASD, the less of a distinction between the self and other conditions, the greater the social symptom severity. A separate DMN study conducted in adults showed the opposite trend in symptom association with RB positively correlating with functional connectivity between the left PCC and the parahippocampal gyrus (Monk et al., 2009). Although developmental differences cannot be ruled out, a simpler explanation may be an anterior-posterior distinction in DMN connectivity. A study measuring structural covariance in the DMN showed the PCC to be correlated with posterior regions of the DMN and with motor, associative, and sensory regions not associated with the DMN in neurotypical individuals, but no correlations were found with prefrontal DMN regions (Zielinski et al., 2012).

Growing evidence has shown that neurotypical processing is characterized not only by synchrony within networks, but by coordination between networks. This may be particularly important for proper inhibitory functioning (Agam et al., 2010) and has found to be decreased between motor and limbic networks (Gotts et al., 2012). The salience network has also shown reduced coordination with frontal-parietal areas in ASD, which are involved in top-down inhibitory processing and include the right middle frontal, inferior frontal, and inferior parietal gyri (Kana et al., 2007). Disrupted salience network functioning, in particular, may provide a plausible link to impaired synchrony between networks in ASD. The default mode network, for example, is reportedly active during ‘resting-state’ yet shows correlations with RB (Monk et al., 2009; Weng et al., 2010). A key prefrontal DMN region, the vmPFC, showed decreased functional connectivity for widespread regions including the anterior and mid-cingulate cortex, premotor cortex, somatosensory cortex, intraparietal sulcus, cerebellum, and several areas in the temporal lobe (Lombardo et al., 2010). One explanation for the connectivity between the ventromedial prefrontal cortex and sensorimotor
regions suggests that embodied cognition, understanding others’ actions, emotions, and sensations, requires a balance of higher order representations at the cognitive level and lower order representations at the sensorimotor level (Lombardo et al., 2010). In ASD, this could manifest as an imbalance of higher-order cognition, related to DMN or SN processing, and lower-order cognition, related to sensorimotor functioning in the striatum. However, the possibility that an imbalance between higher- and lower-order circuitry exists in ASD has not been fully investigated.

**OBJECTIVES AND HYPOTHESES**

The purpose of the current study was to examine brain areas related to RB in ASD by looking at functional connectivity i) for specific structures ii) between structures within two separate networks (DMN and SN) and iii) between networks (DMN with SN). Based on previous findings of striatal abnormalities, one seed in the right dorsal caudate nucleus (dCN) was used to examine striatal connectivity with cortical areas involved in cognitive control. A second striatal seed was placed in the right dorsal caudal putamen (dcP), to assess functional connectivity with sensorimotor areas and examine possible links with RB. Attentional and inhibition-related functioning of the salience network were investigated using a seed in the anterior insula (aI) and another in the dorsal anterior cingulate cortex (dACC) for the right hemisphere. Finally, in order to examine default mode network connectivity, seeds were placed in the left hemisphere in the posterior cingulate cortex (PCC) and in the ventral medial prefrontal cortex (vmPFC). Consistent with previous findings, ASD groups were expected to show overconnectivity for all seeds. Reduced connectivity between seeds within a network were hypothesized for the DMN and SN in ASD. Finally, networks were expected to be less correlated with one another in ASD than in TD. Total RB scores were hypothesized to correlate with overconnectivity of the putamen, reduced connectivity within the DMN and SN, and reduced connectivity between networks. Higher-order RB scores were hypothesized to correlate with overconnectivity of the dCN and reduced connectivity within the SN, given the role of these areas in cognitive control. Since lower-order RB are presumably involved in similar motor pathways as higher-order processing but do not share the cognitive component, no prediction is specific to lower-order RB scores.
CHAPTER 2

METHODS

PARTICIPANTS

Participants consisted of 35 adolescents with ASD between 9 and 17 years and 31 typically developing (TD) adolescents matched on age, gender, IQ, and handedness (Table 1). ASD diagnoses were made based on the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000), the Autism Diagnostic Interview-Revised (ADI-R; Lord, Rutter, & Le Couteur, 1994), and expert clinical judgment according to the DSM-IV-TR (American Psychiatric Association [APA], 2000). The breakdown of specific diagnoses were: 18 participants with Asperger syndrome, 13 with autism spectrum disorder, and 3 participants with Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS). ASD participants were without any co-morbid, autism-related disorders (i.e. epilepsy, fragile X syndrome) and had IQs measuring greater than 70 according to the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 2008), thus meeting inclusion criteria for the study. TD participants were recruited by advertisements in the community and reported no family history of autism, autism-related disorders, or psychiatric conditions. For all participants, handedness was confirmed using the Edinburgh Handedness Inventory (Oldfield, 1971). Informed assent and consent was obtained for all participants and caregivers prior to the study in accordance with San Diego State University and University of California, San Diego institutional review boards.

SCALES AND INSTRUMENTS

RB was assessed using the Repetitive Behavior Scale-Revised (Bodfish, Symons, Parker, & Lewis, 2000; Bodfish, Symons, & Lewis, 1999), a caregiver-report measure consisting of 43 items rating the presence and severity of RB occurring within the past month from 0 (“behavior is not present”) to 3 (“behavior occurs and is a severe problem”). The scale consists of six subscales, which include stereotyped behavior, self-injurious behavior, compulsive behavior, ritualistic behavior, sameness
### Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Group Matching</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASD (n = 35)</td>
<td>TD (n = 31)</td>
</tr>
<tr>
<td>Age</td>
<td>13.55 (2.68)</td>
<td>12.89 (2.46)</td>
</tr>
<tr>
<td></td>
<td>7.67-17.67</td>
<td>8.00-16.58</td>
</tr>
<tr>
<td>Sex (Male:Female)</td>
<td>31:4</td>
<td>24:7</td>
</tr>
<tr>
<td>Handedness (Right:Left)</td>
<td>31:4</td>
<td>25:6</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>104.06 (19.13)</td>
<td>108.08 (11.02)</td>
</tr>
<tr>
<td></td>
<td>70-147</td>
<td>87-133</td>
</tr>
<tr>
<td>Nonverbal IQ</td>
<td>105.91 (17.08)</td>
<td>109.29 (11.18)</td>
</tr>
<tr>
<td></td>
<td>69-140</td>
<td>88-137</td>
</tr>
<tr>
<td>Full-scale IQ</td>
<td>105.91 (17.93)</td>
<td>109.68 (10.59)</td>
</tr>
<tr>
<td></td>
<td>73-141</td>
<td>88-132</td>
</tr>
<tr>
<td>RBS–R Total</td>
<td>31.58 (21.95)</td>
<td>1.39 (2.36)</td>
</tr>
<tr>
<td></td>
<td>1-78</td>
<td>0-7</td>
</tr>
<tr>
<td>RBS–R Cognitive</td>
<td>24.26 (19.04)</td>
<td>1.33 (2.38)</td>
</tr>
<tr>
<td>Subscale</td>
<td>1-72</td>
<td>0-7</td>
</tr>
<tr>
<td>RBS–R Motor</td>
<td>5.79 (5.81)</td>
<td>.06 (.24)</td>
</tr>
<tr>
<td>Subscale</td>
<td>0-25</td>
<td>0-1</td>
</tr>
</tbody>
</table>

Note: Values represented are: mean (SD) range. Significance value, p, for group matching in independent t-tests, for RBS–R total correlations ASD group characteristics.

behavior, and restricted behavior. The first two subscales (stereotyped and self-injurious behaviors) correspond to lower-order sensorimotor RB (Georgiades, Papageorgiou, & Anagnostou, 2010). Example items include “Whole body (body rocking, body swaying)” and “Hits self with body part (hits or slaps head, face, or other body area).” The last four subscales (compulsive, ritualistic, sameness, and restricted behaviors) correspond to higher-order cognitive RB. Example items include “Arranging/ordering (arranges certain objects in a particular pattern or place; need for things to be even or symmetrical)” and “Fascination, preoccupation with one subject or activity (e.g., trains, computers, weather, dinosaurs).” Data were collected at the time of the fMRI scan for most participants while some were
acquired from 6 months to 3 years after the scan. A cumulative score and two subscale scores (lower-order and higher-order) were calculated for each participant.

To more comprehensively assess ASD characteristics, specifically those most closely associated with RB, additional parent-report measures were acquired including the Caregiver Sensory Profile (SP; Dunn, 1994), the Social Responsiveness Scale (SRS; Constantino & Gruber, 2002), and the Behavior Report Inventory of Executive Functioning (BRIEF; Gioia, Isquith, Guy, & Kenworthy, 2000). The SP consisted of four subscales measuring the frequency of behaviors related to sensory processing with higher scores reflecting more typical behavior. The SRS used 5 subscales to measure a breadth of behavior reported to be atypical in ASD, including social awareness, communication, motivation, cognition, and mannerisms. Finally, the BRIEF provided an assessment of executive functioning using 8 subscales, 2 behavioral indices, and 1 global composite score, and was included to help elucidate the relationship between RB and executive functioning.

**MRI Data Acquisition**

Brain imaging data was acquired on a 3T GE Scanner using an 8-channel head coil. High resolution anatomical data was obtained using a standard T1 weighted sequence (TR = 11.08 ms; TE = 4.3 ms; field of view [FOV] = 256 mm; 256 x 256 matrix; 180 slices; flip angle = 45°; 1 mm³ resolution). Resting state functional data was acquired for 185 time points using T2* weighted imaging with a single-shot gradient-recalled, echo-planar pulse sequence (TR = 2000 ms; TE = 30ms; FOV = 220 mm; flip angle = 90°; 64 x 64 matrix; 3.438 mm² in-plane resolution). The session lasted 6 minutes and 10 seconds, during which participants were presented with a white fixation cross displayed on a black background and instructed, “keep your eyes on the cross, relax, let your mind wander, and try not to fall asleep.”

**fMRI Data Preprocessing**

Data were preprocessed and analyzed using the Analysis of Functional Neuroimaging suite (AFNI; Cox, 1996). The first five time points were discarded to allow for magnetic field stabilization. The remaining 180 time points were field-map corrected to minimize error due to magnetic field inhomogeneity, slice-time corrected for interleaved acquisition, and motion corrected by realigning whole volume data to the first time point. Functional data were then
co-registered on to anatomical images, resampled to 3.0 mm isotropic voxels, standardized to N27 Talairach space (Talairach & Tournoux, 1988), and spatially blurred with an isotropic Gaussian filter to a full-width at half-maximum (FWHM) of 6 mm. Finally, a band-pass filter of $0.008 < f < 0.08 \text{ Hz}$ was be applied to restrict frequencies to an optimal range for detecting network-specific correlations in BOLD fluctuations (Fox & Raichle, 2007).

Additional motion correction procedures were performed to reduce measurement error associated with head motion on BOLD signal correlations. Six rigid-body motion parameters (three rotations, three translations) including their derivative were modeled as regressors and removed from the data. Time points of excessive head motion ($> 0.75\text{mm relative to the previous time point}$) were censored, and segments with fewer than 10 consecutive surviving time points were discarded. Additionally, the average signals for white matter and ventricular areas along with their derivatives were obtained using Freesurfer software (Fischl et al., 2004) and removed from the data. Physiological (heart rate, respiration) time series were also removed through linear regression. Comparisons between TD and ASD groups for percentage of data censored and total motion were made to evaluate group differences in data quality.

**Regions of Interest**

A meta-analysis of 126 striatal functional connectivity studies supports rostral/caudal and dorsal/ventral connectivity gradients within the striatum (Postuma & Dagher, 2006). In the putamen, the rostral portion shares connections with executive prefrontal regions while the caudal portion has connections with primary motor cortical areas. Additionally, dorsal portions of the caudate and putamen are connected with cognitive control regions and motor systems whereas ventral portions are connected with limbic structures. Based on findings of striatal abnormalities within ASD (Di Martino et al., 2011), a seed was placed in the dorsal caudal putamen (dCP) to examine connectivity with primary motor areas, and a second seed, placed in the dorsal caudate nucleus (dCN), examined connectivity with cognitive control areas. To examine connectivity of the default mode network, seeds were placed in the ventromedial prefrontal cortex (vmPFC) and the posterior cingulate cortex (PCC). Finally, cognitive control functioning and network switching was examined with two seeds in the salience network, including the anterior insula (AI) and dorsal anterior cingulate cortex.
(dACC). The default mode network seeds were placed in the left hemisphere with all other seeds in the right hemisphere. Notably, the striatal ROIs were the only ones not nested within a functional network due to their connectivity with nonoverlapping areas. Spherical masks were drawn (6mm radius) using coordinates based on previous studies (Table 2)

Table 2. Talairach Coordinates of Regions of Interest

<table>
<thead>
<tr>
<th>Region</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>dcPut</td>
<td>28</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>dCN</td>
<td>13</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>vmPFC</td>
<td>-2</td>
<td>42</td>
<td>-8</td>
</tr>
<tr>
<td>PCC</td>
<td>-5</td>
<td>-49</td>
<td>40</td>
</tr>
<tr>
<td>aIns</td>
<td>36</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>dACC</td>
<td>11</td>
<td>21</td>
<td>26</td>
</tr>
</tbody>
</table>

**FCMRI Data Analysis**

Once masks were drawn, the average BOLD time series was extracted from each seed for each participant. Mean time series for each seed were then correlated with the time courses of all other voxels in the brain in a whole-brain voxel-wise analysis using Pearson’s correlations. Correlation coefficients were transformed to normally distributed values using Fisher’s r-to-z transformation. One- and two-sample t-tests examined within- and between-group effects of functional connectivity. Statistical maps were set to a cluster-corrected threshold of \( p < .05 \) to correct for multiple comparisons (Forman et al., 1995). Correlations between the mean time courses of each seed pair were calculated and transformed into standardized z scores, resulting in an 8 x 8 correlation matrix. These values were entered into a group level analysis using a t-test to detect between-group differences. Correlation values of seeds within the salience network were correlated with correlation values of seeds within the default mode network to assess between network coordination separately for ASD and TD groups.

To examine the relationship between neural abnormalities and repetitive behavior symptoms, functional connectivity was correlated with repetitive behavior scores at three levels within ASD: individual seeds, seeds within a network, and correlations between networks. Clusters showing significant group differences in functional connectivity were
correlated with cumulative RB scores for each seed. Correlations among mean time course ($z$) for seeds within each respective network were correlated with cumulative RB scores. Finally, the average between-network correlation was correlated with cumulative RB scores.
CHAPTER 3

RESULTS

A whole-brain functional connectivity analysis revealed similar patterns of connectivity for ROIs in TD and ASD groups with BOLD correlations occurring among regions of known functional networks, including frontal parietal areas with the SN, medial and superior frontal areas with the DMN, the sensorimotor cortex with the putamen, and the prefrontal cortex with the caudate nucleus. In a direct group comparison, however, several clusters of significantly different connectivity emerged between groups. All seeds showed mixed over- and underconnectivity for the ASD group relative to the TD group, except for the SN and dCN, which were exclusively underconnected in the ASD group.

The SN mask consisted of two ROIs – the AI and the dACC- in which time series were averaged across all voxels to assess network connectivity as well as separately for AI and dACC to assess ROI specific connectivity. As a whole, the SN showed reduced connectivity with a small area of the anterior prefrontal cortex, bilaterally, and with the left cerebellum extending into the right cerebellum and brainstem (Figure 1A). AI and dACC connectivity patterns closely corresponded to the network as a whole with the AI showing additional underconnectivity with the left inferior temporal gyrus and left temporal pole. Overconnectivity was also seen for individual AI and dACC seeds with the right inferior parietal lobule and right somatosensory cortex, respectively.

The DMN mask was drawn similarly to the SN by averaging across all voxels in the PCC and mPFC; these nodes were also examined independently. The network mask showed decreased within-network connectivity with dissociable areas of the mPFC and PCC, the left angular gyrus and the medial cerebellum. Analyses of each DMN region, independently, yielded mPFC underconnectivity with the left angular gyrus, medial cerebellum, and brainstem, and PCC underconnectivity with areas of the PCC and the left superior temporal gyrus. Overconnectivity was also found for the network mask and its individual ROIs (Figure 1B). In particular, connectivity differences were found for the DMN network with the left ventral premotor cortex, the anterior insula, the bilateral frontal eye fields, the
Figure 1. Significant clusters of functional connectivity effects between TD and ASD groups for: (A) Salience network (SN). (i) Anterior insula (AI). (ii) Dorsal anterior cingulate cortex (dACC). (B) Default mode network (DMN). (i) Medial prefrontal cortex (mPFC). (ii) Posterior cingulate cortex (PCC). (C) Dorsal caudal putamen (dcP). (D) Dorsal caudate nucleus (dCN). All clusters shown are significant (p < .05 corr.). Yellow represents positive t-scores (i.e., TD < ASD); blue represents negative t-scores (i.e., TD > ASD).
superior and inferior parietal lobules, middle occipital gyrus, precuneus, and inferior frontal gyrus. The PCC ROI drove these results showing overconnectivity with all DMN clusters and additional overconnectivity with lateral cerebellum, anterior prefrontal cortex, and right ventral premotor cortex. The mPFC, meanwhile, showed overconnectivity exclusively with the premotor cortex.

Reduced connectivity with regions involved in corticostriatal pathways was seen in the basal ganglia ROIs. The putamen typically receives input from all areas of the sensorimotor cortex and projects back to more localized areas of the primary and secondary cortical motor areas (Alexander, 1986; Di Martino et al., 2008). In ASD, however, underconnectivity was found for the left ventral premotor cortex and posterior regions of the left middle temporal gyrus (Figure 1C). The caudate nucleus was underconnected in ASD with the dorsolateral prefrontal cortex, the right temporo-parietal junction, and left precuneus (Figure 1D). A sole region of overconnectivity for the putamen seed was found in the rostral ACC.

Functional connectivity among seed regions was assessed through an 8 x 8 correlation matrix. For both groups, the anterior insula and dorsal anterior cingulate seeds of the SN were highly correlated, while the posterior cingulate cortex and medial prefrontal cortex seeds of the DMN showed only minimal correlation for both groups. The putamen was moderately connected with the AI and dACC and minimally with the mPFC and PCC, consistent with other findings (Di Martino et al., 2008). The caudate nucleus showed only modest correlations with other ROIs. Between-group t-tests for each ROI pair failed to reveal any significant differences for either within-network or between-network connectivity for the DMN and SN. However, significantly increased connectivity was found between the putamen and caudate nucleus ROIs in the ASD group ($F(1,65) = 4.70, p = .034$, uncorr.).

**RBS–R Correlation with Severity and Demographics**

As expected, the ASD group showed greater symptom severity on the RBS–R than the TD group ($F = 33.63, p < .001$). Notably, RBS—R scores were available for only a subset of the overall sample ($N = 19$ ASD, 18 TD). While the distribution of TD scores indicated a floor effect (Table 1), the ASD group showed sufficient variability and relatively normal distribution of RBS–R scores to warrant further within-group analyses. In ASD,
RBS–R total scores did not correlate with age, sex, or handedness, but did correlate with full-scale IQ ($r = - .54, p = .022$) and verbal IQ ($r = - .53, p = .023$), with fewer RB symptoms reported for those with higher IQs. RBS–R cognitive subscales showed similar pattern of correlations (FSIQ: $r = - .63, p = .005$; VIQ: $r = - .64, p = .005$; NVIQ: $r = - .53, p = .018$), whereas RBS–R motor subscales did not ($p > .90$). Therefore, full-scale IQ was entered as a covariate into all correlations for RBS–R total and cognitive scores.

Further, correlations were calculated between the RBS–R and diagnostic measures: the ADI and ADOS, as well as related symptom severity measures: Caregiver Sensory Profile (SP), the Social Responsiveness Scale (SRS), and the Behavior Rating Inventory of Executive Function (BRIEF). Overall, the RBS–R showed the strongest correlations with qualitatively similar scales, including the SRS and the SP, most notably for ‘sensation seeking’ (Table 3). Diagnostic measures correlated with the RBS–R at significant or marginally significant levels, except for the ADOS RB subscale, which unexpectedly did not ($p > .40$). Fewer correlations were seen on the measure of executive functioning, which has shown null findings with RB or only partial overlap of RB with various aspects of executive functioning (Boyd et al., 2009; Lopez, Lincoln, Ozonoff, & Lai, 2005).

**FC CORRELATES OF RB**

Several ROI pairs were significantly correlated with RBS–R scores within ASD. In particular, as connectivity between the dcP and DMN increased, RBS–R cognitive scores decreased ($r = - .48, p = .009$) (Figure 2). Individual DMN seeds contributed relatively equally to this effect (mPFC: $r = - .46, p = .013$; PCC: $r = - .39, p = .043$). Negative correlations were also found with RBS–R cognitive scores being associated with increased connectivity between the AI and dCN ($r = - .40, p = .035$) and marginally for connectivity between the AI and dcP ($r = - .37, p = .055$). RB motor scores showed no correlation with ROI pairs. There was also a marginally significant correlation, in the predicted direction, for lower RBS–R total scores to correspond with higher connectivity between the SN and DMN ($p = .13$). When this network pair was correlated with SRS Cognition scores using a larger sample size, a significant negative correlation emerged ($r(33) = - .567, p < .001$; Figure 3).
Table 3. RBS–R Correlations with Diagnostic and Behavioral Measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>r(18)</th>
<th>p</th>
<th>Measure</th>
<th>r(18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADI</td>
<td></td>
<td></td>
<td>SRS (cont.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soc</td>
<td>.48</td>
<td>.043</td>
<td>Motivation</td>
<td>.62</td>
<td>.006</td>
</tr>
<tr>
<td>Com</td>
<td>.45</td>
<td>.059</td>
<td>Autistic mannerisms</td>
<td>.81</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>RB</td>
<td>.57</td>
<td>.013</td>
<td>Total</td>
<td>.67</td>
<td>.003</td>
</tr>
<tr>
<td>ADOS</td>
<td></td>
<td></td>
<td>BRIEF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soc</td>
<td>.42</td>
<td>.070</td>
<td>Inhibit</td>
<td>.56</td>
<td>.025</td>
</tr>
<tr>
<td>Com</td>
<td>.80</td>
<td>&lt;.001</td>
<td>Shift</td>
<td>.64</td>
<td>.007</td>
</tr>
<tr>
<td>RB</td>
<td>.20</td>
<td>.42</td>
<td>Emotional control</td>
<td>.42</td>
<td>.104</td>
</tr>
<tr>
<td>SP</td>
<td></td>
<td></td>
<td>Initiate</td>
<td>.32</td>
<td>.224</td>
</tr>
<tr>
<td>Low regulation</td>
<td>-.56</td>
<td>.016</td>
<td>Working memory</td>
<td>.40</td>
<td>.120</td>
</tr>
<tr>
<td>Sensory sensitivity</td>
<td>-.67</td>
<td>.003</td>
<td>Plan/Organize</td>
<td>.31</td>
<td>.250</td>
</tr>
<tr>
<td>Sensation seeking</td>
<td>-.85</td>
<td>&lt;.001</td>
<td>Organization of materials</td>
<td>.19</td>
<td>.48</td>
</tr>
<tr>
<td>Sensory avoidance</td>
<td>-.62</td>
<td>.006</td>
<td>Monitor</td>
<td>.54</td>
<td>.032</td>
</tr>
<tr>
<td>SRS</td>
<td></td>
<td></td>
<td>Behavioral regulation index</td>
<td>.67</td>
<td>.005</td>
</tr>
<tr>
<td>Awareness</td>
<td>.57</td>
<td>.014</td>
<td>Metacognition index</td>
<td>.33</td>
<td>.208</td>
</tr>
<tr>
<td>Communication</td>
<td>.50</td>
<td>.036</td>
<td>Global executive composite</td>
<td>.55</td>
<td>.027</td>
</tr>
<tr>
<td>Cognition</td>
<td>.66</td>
<td>.003</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Significance values are uncorrected. Items on the SP are reverse scored relative to other scales. ADI (Autism Diagnostic Interview); ADOS (Autism Diagnostic Observation Schedule); SP (Caregiver Sensory Profile); SRS (Social Responsiveness Scale); BRIEF (Behavior Rating Inventory of Executive Function).

From the whole-brain connectivity analysis, increased RBS–R scores (Table 4) were largely associated with: (1) underconnectivity between functionally related regions and (2) overconnectivity with outside-network regions. PCC underconnectivity with the left superior temporal gyrus, an area implicated in default mode function and social cognition, was correlated with RBS–R total scores ($r = -.48, p = .017$). This effect was driven by RB motor scores ($r = -.69, p = .001$) rather than RB cognitive scores ($r = -.35, p = .07$). RB motor scores were also marginally associated with underconnectivity between the anterior prefrontal cortex ($r = -.45, p = .052$). RB cognitive scores were correlated with underconnectivity of the dACC with the left middle temporal gyrus ($r = -.40, p = .035$).
Figure 2. Relationship between RBS–R Cognitive subscale and connectivity (z') for: (A) dcP x DMN (r(18)=-.48, p=.009), (B) dcP x mPFC (r(18)=-.46, p=.013), and (C) dcP x PCC (r(18)= -.39, p=.043).
Figure 3. Relationship between SN x DMN connectivity ($z'$) and SRS Cognition subscales within ASD ($r(33)=-.567, p<.001$).

Additionally, underconnectivity of the dcP with the left ventral premotor cortex ($r = -0.37, p = 0.054$) approached significance with RBS–R cognitive scores as well as for dCN underconnectivity with the right DLPFC ($r = -0.34, p = 0.076$). Related behavioral measures were consistent with many of these findings and showed additional correlations. Increased symptom severity was related to underconnectivity for the dACC with the right temporo-parietal junction for SRS Cognition ($r = -0.42, p = 0.014$) and Autistic mannerisms ($r = -0.40, p = 0.019$) scores. This effect was also present within the TD group: SRS Cognition ($r = -0.49, p = 0.006$), Autistic Mannerisms ($r = -0.41, p = 0.023$). Underconnectivity between the mPFC and brainstem also correlated with all four measures of the SP, most significantly for sensory avoidance ($r = -0.56, p = 0.004$). More impaired functioning corresponded to greater overconnectivity of the PCC with executive control areas. This was found, in particular, for areas of left lateral cerebellum, implicated in error-processing, and was correlated with increased severity on SP sensory seeking ($r = -0.42, p = 0.035$) and ADI RB scores ($r = 0.36, p = 0.012$) with the right thalamus.
Table 4. Associations Between RBS–R scores and Clusters Showing Group Connectivity Differences

<table>
<thead>
<tr>
<th>ASD &gt; TD</th>
<th>RBS–R Total</th>
<th>Peak Coordinates (Talairach)</th>
<th>Cluster size (# Voxels)</th>
<th>t-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r(18)</td>
<td>p</td>
<td>x</td>
<td>y</td>
</tr>
<tr>
<td>PCC x L STG</td>
<td>.48</td>
<td>.017</td>
<td>-48</td>
<td>-27</td>
</tr>
</tbody>
</table>

**RBS–R Cognitive**

|         | r(18) | p | x | y | z |         |
| PCC x L STG | .35 | .067 | -48 | -27 | 24 | 55 | 3.36 |
| dCN x R DLPFC | .34 | .076 | 44 | 23 | 38 | 158 | 4.33 |
| dcP x L MTG | .40 | .035 | -45 | -66 | 15 | 119 | 3.53 |
| dcP x L vPMC | .37 | .054 | -57 | -12 | 34 | 140 | 3.74 |

**RBS–R Motor**

|         | r(18) | p | x | y | z |         |
| PCC x L STG | .69 | .001 | -48 | -27 | 24 | 55 | 3.36 |
| dACC x L aPFC | .53 | .031 | -36 | 48 | 15 | 85 | 4.03 |

Note: Significance values, p, uncorr. STG (Superior temporal gyrus), DLPFC (Dorsolateral prefrontal cortex), MTG (Middle temporal gyrus), vPMC (Ventral premotor cortex), aPFC (Anterior prefrontal cortex).

.041). Overconnectivity between the PCC and left precuneus was also associated with increased ADI-RB scores (r = .51, p = .002).

**Preprocessing Effects**

Due to the potential effects of head motion in creating artifactual group differences (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012; Van Dijk, Sabuncu, & Buckner, 2012), a relatively strict motion threshold of 0.75mm was set for censoring of time points. To ensure reliability of functional connectivity results, data were re-examined at an even stricter motion censoring threshold of 0.25mm, which yielded groups with 24 ASD and 22 TD participants. Five additional participants were excluded from the TD group to match groups based on sex. At this level, results from the correlation matrix were replicated and substantial overlap was seen for significant clusters, except for the basal ganglia. A number
of additional clusters were also found for the AI, which was underconnected with prefrontal
and sensorimotor areas, and the PCC, which showed exclusive overconnectivity, most
extensively in the bilateral occipital cortex. However, these additional clusters did not
substantially alter the trend for overconnectivity to occur between networks, as with the
DMN, and underconnectivity to occur between nodes of a network, as with the AI. Although
fewer correlations between functional connectivity and symptom measures emerged as
significant, the trends in the correlations appeared consistent. This may partly be due to a
loss of statistical power with a smaller sample size.

Data were also re-analyzed using global signal regression (GSR) and its derivative.
This variable is thought to allow for the detection of inverse correlations by extracting the
global signal as a nuisance variable. However, GSR has also been shown to introduce a risk
of distorting group differences (Saad et al., 2012). Nevertheless, findings from a GSR
analysis overlapped closely with those from the original analysis, but with many more
significant clusters, particularly with the dACC, which was overconnected with sensory areas
and the mPFC, which was overconnected with sensorimotor and frontal parietal inhibition
areas. Additionally, the RBS–R correlations with underconnectivity between the dACC and
the anterior prefrontal cortex and between the PCC and superior temporal gyrus were
replicated.

Finally, potential confounding effects of demographic variables and remaining head
motion were controlled for when correlating between behavior and functional connectivity.
Age-dependent variables, which are inherent to developmental research, may be particularly
problematic due to their associations with measures of connectivity as well as behavior. In
the functional connectivity and subsequent analyses, group comparisons were made to ensure
that differences that arose between ASD and TD were not likely due to demographics (age,
sex, handedness, nonverbal IQ) or head motion during the scan (root mean square of
displacement, 6 rigid-body motion parameters). Following group comparisons, data for one
participant was excluded to create a more balanced male:female ratio. In correlation
analyses, symptom severity measures were individually correlated with the same
demographic and motion variables in two separate analyses. Verbal and non-verbal IQ were
positively correlated with total RBS–R scores and cognitive RBS–R scores, therefore, full-
scale IQ was used as a covariate in all RBS–R correlations. The same steps were taken for
other behavioral measures used, including ADI, ADOS, SP, SRS, BRIEF, although these showed no correlation with demographics and no consistent differences in motion.
CHAPTER 4

DISCUSSION

Functional connectivity was assessed from resting-state data using eight ROIs previously associated with RBs in ASD. At a higher level of complexity, we examined two complex network ROIs, i.e. the SN and DMN, each consisting of spatially distinct regions belonging to a common functional network. At a lower level of complexity, we also investigated individual ROIs participating in their respective networks, i.e. the AI and dACC, and for regions not involved in a network, i.e. the dCN and dcP. In accordance with the aims of the study, functional connectivity was examined: (i) within each network, (ii) between one network and the other, and (iii) with regions outside both networks.

WITHIN NETWORK

Regions within a functional network, i.e., SN and DMN, were expected to show decreased correlations with one another in the ASD compared to the TD group. Although the correlation matrix did not show underconnectivity between the AI and dACC seeds nor between the mPFC and PCC seeds, the whole brain analysis yielded underconnectivity within both networks. For the SN, underconnectivity was seen in an area of the right anterior prefrontal cortex, an area which is thought to be a domain-specific extension of the SN, contributing to stable task maintenance, the formation of a core task-set system, and network “switching” (Dosenbach et al., 2006; Seeley et al., 2007). This effect was driven by the dACC, for which underconnectivity was found with the anterior prefrontal cortex, bilaterally, and was, in turn, correlated with RBS—R motor scores for the left hemisphere cluster. Correlations with the RBS—R, however, were expected for the cognitive scores rather than the motor scores. One potential explanation may be the inclusion of varying ASD diagnosis in our sample. Previous findings of reduced connectivity in the SN were reported specifically for individuals diagnosed as having ASD compared to both those with Asperger syndrome and those who were typically developing (Ebisch et al., 2010). Therefore, reduced
connectivity may have been driven by lower-functioning ASD participants within our sample, who in turn, may be expected to show more lower-order RB.

In accordance with previous findings in adolescents with ASD (Assaf et al., 2010; Weng et al., 2010), underconnectivity was also found for the DMN seeds with functionally related areas, including the left angular gyrus and areas of the mPFC and PCC, which were proximal but not overlapping with ROIs comprising the DMN. Likewise, the PCC, when examined individually, showed reduced connectivity with an additional cluster implicated in the DMN, namely the left superior temporal gyrus (Kennedy & Courchesne, 2008). Underconnectivity between the PCC and this cluster, furthermore, correlated with RBS—R total and motor scores, and marginally with RBS—R cognitive scores. This suggests reduced integrity of the network, as a whole, and for the PCC seed, specifically.

Altered DMN connectivity may have implications for the network’s ability to modulate activation between resting state and task activation. In typical development, activity is suppressed in the DMN when goal-oriented behavior is initiated (Kennedy & Courchesne, 2008). Frontal parietal areas of the executive control network (ECN), on the other hand, activate in response to task initiation and are typically inversely correlated with the DMN (Sridharan, Levitin, & Menon, 2008). In ASD, the DMN deactivation response is impaired, implying that network activity remains relatively constant regardless of situational demands (Kennedy, Redcay, & Courchesne, 2006) and that the balance between the opposing DMN and ECN may be disrupted (Uddin & Menon, 2009). In the current study, overconnectivity of the DMN occurred with regions outside this network, primarily with those implicated in the ECN, including the inferior parietal lobule, superior parietal lobule, bilateral frontal eye fields, and inferior frontal gyri (Dosenbach et al., 2006; Fox et al., 2005). This suggests that the DMN and ECN, are not differentially activated in ASD. To a lesser extent, overconnectivity in the DMN was also seen with sensorimotor regions, including the bilateral ventral premotor cortex. Intrinsic correlation patterns show that sensory and motor areas, in typical development, are functionally segregated from the DMN (Fox et al., 2005), which is hypothesized to be a critical feature for optimal functioning of systems involved in unrelated domains (Posner & Peterson, 1990).

Taken together, our results suggest that impairment in ASD extends to large-scale functional networks both in terms of reduced integration within networks and reduced
segregation between networks, which has been shown to characterize ASD neurological profiles more accurately than general underconnectivity (Shih et al., 2011; Rudie et al., 2012). To some extent, this pattern in ASD reflects immature brain functioning in typical development, in which anatomically neighboring regions are highly connected. Gradually, this local connectivity is reduced as connectivity among long-distance, functionally related regions is strengthened (Fair et al., 2007; Stevens, Pearlson, & Calhoun, 2009). The resulting functional specialization, according to the interactive specialization view, arises from genetic influences and environmental interaction, which causes the brain to adapt increasingly more efficient processing styles (Johnson, 2003). However, some evidence in ASD suggests that brain organization does not merely reflect developmental delay, but rather qualitatively altered structure (Langen et al., 2009; Schumann et al., 2010) and function (Di Martino et al., 2011). The same interactive specialization view posits that abnormality in early childhood, as is seen in ASD, consists of a core impairment which compromises subsequent processing of the environment and, ultimately, leads to different patterns of organization (Johnson et al., 2003).

**Between Network**

The interaction between the DMN and SN and its relationship to symptom severity was of particular interest given the paucity of research linking ASD symptoms to connectivity between large-scale networks. Previous findings have shown reduced connectivity between the SN and an executive control system in ASD (Kana et al., 2007). Due to the putative role of the SN in shifting attentional resources between large-scale networks (Seeley et al., 2007), reduced connectivity of the SN with large-scale networks is thought to indicate disrupted processing of network functions and, consequently, impaired synchronization of functional networks. Although no group difference in connectivity between the SN and DMN emerged in our sample, which consisted predominantly of high-functioning individuals with ASD, a significant relationship within ASD was found for between-network connectivity with symptom severity. This occurred in the predicted direction with greater connectivity between the SN and DMN being associated with fewer symptoms on the SRS cognition score. We also found a marginal correlation for the RBS–R cognitive score with connectivity between the SN and mPFC, a canonical DMN node, which
was, however, only marginally significant, possibly due to the smaller sample size for RBS analyses. Taken together, these findings are consistent with a systems-level model emphasizing the importance of the SN in facilitating synchronous network interaction (Uddin & Menon, 2009). Notably, resting state data have yielded similar findings of spontaneous shifts occurring between the internally-directed DMN and the externally-oriented ECN (Sridharan et al., 2008). Results, therefore, suggest that increased symptom severity in ASD may be associated with impaired synchronization between the SN and DMN.

**OUTSIDE NETWORK**

Finally, connectivity between basal ganglia and large-scale functional networks has not, as yet, been studied systematically although it may have important implications for RBS symptoms. The relationship between severity scores and connectivity patterns in the putamen and caudate nucleus were characterized by two general findings, in which increased symptom severity corresponded to: (1) reduced connectivity between basal ganglia ROIs and regions involved in corticostriatal pathways, and (2) reduced connectivity between basal ganglia ROIs and DMN regions. The putamen and the caudate nucleus have been shown to belong to anatomically and functionally segregated corticostriatal loops projecting to sensorimotor areas and prefrontal cortical areas, respectively (Alexander et al., 1986; Di Martino et al., 2008). Underconnectivity in the sensorimotor pathway, specifically between the dcP and left middle temporal gyrus as well as the putamen and left ventral premotor cortex (marginally), was correlated with RBS–R cognitive scores. In the prefrontal associative pathway, the caudate nucleus has been shown to correlate with frontal parietal regions supporting cognitive control, including the DLPFC and right temporo-parietal junction (Di Martino et al., 2008; Dosenbach et al., 2006), both of which were underconnected with the dCN in ASD; underconnectivity for both regions was, in turn, marginally correlated with RBS–R cognitive scores. Previously, the same striatal ROIs have shown connectivity in ASD with areas lacking connectivity or inversely correlated in TD, such as those involved in sensory integration, including the supramarginal gyrus, fusiform gyrus, and cuneus (Di Martino et al., 2011). Although the present study did not reproduce these findings of aberrant striatal connectivity, overconnectivity with functionally-disparate regions, as previously reported, is not at odds with the current findings of underconnectivity.
with functionally related areas comprising corticostriatal loops. Immature or abnormal brain development can be characterized both by reduced connectivity of long-distance functional networks and by increased connectivity with regions outside a functional network. Therefore, the current findings may complement previous findings by implicating the striatum in additional patterns of impaired network-related functional connectivity (Di Martino et al., 2011). Additionally, increased connectivity of the putamen with the DMN as a whole and individual PCC and mPFC seeds was associated with fewer RB symptoms. This, again, suggests that increased BOLD correlation between the basal ganglia and cortical regions is associated with more adaptive functioning in ASD.

Overall, results suggest that both overconnectivity and underconnectivity contribute to RB symptoms. This partially aligns with previous findings; however, a greater degree of underconnectivity in the basal ganglia and overconnectivity in the DMN were found compared to other recent, similar studies. Inconsistencies in the literature may be due, in part, to methodological factors. In particular, underconnectivity is most commonly reported for studies whose designs include task effects, whereas those using task regression or resting-state data have tended to show mixed results (Müller et al., 2011). Similarly, underconnectivity tends to predominate in the absence of low-pass or band-pass filtering and may also receive more focus than overconnectivity when the field of view is limited to ROIs rather than the whole brain (Nair, in press). Current findings appear most consistent with other studies using whole-brain analyses to measure intrinsic connectivity. Additionally, patterns of connectivity could be characterized, in large part, by the differentiation of functional networks. Useful indices of network connectivity include network integrity and network segregation, both of which appeared to be related to ASD symptoms. Therefore, investigating brain networks and their interactions on a systems-level may facilitate a more comprehensive understanding of altered brain processing in ASD and associated behaviors.

LIMITATIONS

Several steps throughout the data collection and analyses could be refined in future studies to allow greater sensitivity to between-group differences. For the current study, there was a variable lag between participants’ fMRI data acquisition and RBS–R administration with the longest time difference being two years for three participants; however, for most,
imaging data and questionnaires were completed at the same time. This is important because RB has been shown to decrease with age while developmental changes occurring during childhood and adolescence create a rapidly changing brain landscape. Data, therefore, may have incurred some loss in sensitivity, particularly to the correlation between behavioral and brain imaging measures.

Taking into consideration the subtypes of diagnoses may also improve the sensitivity of data analyses. In particular, an area in the SN has been shown to differ between those with Asperger syndrome and those with ASD. Controlling for diagnoses or symptom severity may reveal additional differences in connectivity between subgroups. Likewise, future studies may take into account more qualitative components of repetitive behavior, including the context in which they occur (i.e. negative vs. positive situations). The RBS–R showed high correlations with the diagnostic and other behavioral measures, yet it proved to be the most sensitive in correlating with functional connectivity measures, even with a smaller sample size than related behavioral measures, which had a larger sample size. This increased sensitivity may be due to the breadth of behaviors assessed with the RBS–R. When tested using a larger sample size, the RBS–R may be particularly instrumental in discerning between types of RB (motor vs. cognitive) and moving toward characterizing individual ASD subtypes.

The current study is the first to explore abnormal functional connectivity in ASD in relation to RB symptoms using a psychometrically validated scale specifically designed to do so. RB continues to be a prevalent symptom of ASD though its link with sociocommunicative symptoms remains largely unknown. Identifying this link and understanding the role of RB in ASD will be important for characterizing the disorder neurologically, especially as recent changes in diagnostic criteria place increased emphasis on RB symptoms, giving both breadth and depth of RB symptoms greater weight among the three core characteristics of ASD (APA, 2013).
REFERENCES


