ALTERATIONS IN FUNCTIONAL CONNECTIVITY OF NEURAL NETWORKS IN ADOLESCENTS WITH AUTISM SPECTRUM DISORDERS

by

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To Pa and Ma,

Who brought me into this world to learn, to love and to laugh…

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ABSTRACT

ALTERATIONS IN FUNCTIONAL CONNECTIVITY OF NEURAL NETWORKS IN ADOLESCENTS WITH AUTISM SPECTRUM DISORDERS

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The present set of studies sought to examine functional connectivity in adolescents with autism spectrum disorders (ASD) using functional magnetic resonance imaging (fMRI). In Chapter II, functional connectivity was examined in the context of a social task with emotional faces. The task was designed to control for attention differences between groups as well as to elicit robust amygdala activation in both groups. Using a psychophysiological interaction (PPI) analytic technique, we examined relationships between the amygdala and various cortical structures associated with face processing. The results showed that although behavioral performance did not differ between groups, adolescents with ASD relative to controls, showed weaker positive amygdala-cortical connectivity within the temporal and frontal regions when viewing emotional faces relative to baseline. In addition, weaker positive connectivity was associated with the degree of social impairment in the ASD group. The findings suggest that adolescents with ASD show a disruption in functional connectivity in the neural

networks involved in face processing and that these disruptions relate to social impairment, and are not driven by behavioral differences in emotion recognition. In Chapter III, functional connectivity within the default network was examined using a seed in the posterior cingulate cortex (PCC) in the absence of a cognitive task (subjects lay in the MRI and viewed a cross on a screen). The default network is of interest as others have identified it as the brain's intrinsic activation, important in maintaining the equilibrium between excitatory and inhibitory neuronal inputs and low-level monitoring of the external surroundings. The results of the resting connectivity study revealed that relative to controls, adolescents with ASD showed weaker connectivity between the PCC and a majority of areas within the default network, with the exception of tighter connectivity between the PCC and the right superior temporal gyrus. Moreover, poorer adaptive behavior was related to weaker connectivity between the PCC and left angular gyrus in adolescents with ASD. To summarize, the present set of studies show evidence for widespread reduced connectivity and isolated areas of increased connectivity in adolescents with ASD relative to controls.

CHAPTER I

INTRODUCTION

Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by deficits in social and communicative functioning, in the presence of restricted and repetitive behaviors and interests (RRBs). Afflicting as many as 1 out of 166 children, ASD has a devastating impact on the individual, family and society (Charles, Carpenter, Jenner, & Nicholas, 2008). Although ASD can only be defined behaviorally, neuroimaging research has made strong contributions to identifying specific brain regions that are implicated in ASD. One region that has received considerable attention is the amygdala. Its involvement in social and emotional processing has spearheaded models that link impairments seen in ASD to dysfunction within the amygdala (Baron-Cohen, et al., 2000; Schultz, 2005). The field of ASD is now advancing beyond localized models into examining neural networks that are disturbed within ASD (Muller, 2008). Recently, there have been a growing number of studies that emphasize that disruption in ASD is a function of abnormal connectivity rather than dysfunction within a specific region of the brain (Belmonte, et al., 2004; Wickelgren, 2005). Therefore, examining interrelationships between brain regions using functional connectivity methods can provide useful information about how activity in one region correlates to another in the presence of a cognitive task or during resting state.

To date, there remains no conclusive evidence for specific biological markers that

can confirm a diagnosis of ASD. However, family and twin studies provide substantial evidence that genetics plays a key role in determining the etiology of this disorder (Folstein & Rosen-Sheidley, 2001). Twins studies indicate that concordance rates for ASD are much higher in monozygotic twins (range: 82% to 92%) than in dizygotic twins (range: 1% to 10%) (Persico & Bourgeron, 2006). In addition, siblings, parents and relatives of ASD probands have been reported to exhibit personality traits that are similar, albeit less severe in nature to the social deficits seen in ASD probands (Dawson, et al., 2007).

Progress is being made to understand the multitude of genes that confer a risk for ASD. Of these studies, many have focused on the identification of genes that result in disturbances in neural development (Belmonte & Bourgeron, 2006; Bourgeron, 2007). First, cell adhesion molecules known as neuroligins play a role in maintaining the balance between inhibitory and excitatory signals (Chih, Engelman, & Scheiffele, 2005). Reports of mutations in neuroligin 3 and neuroligin 4 genes have led to synaptic abnormalities within ASD (Persico & Bourgeron, 2006). Second, lower levels of reelin proteins, which facilitate neuronal migration during prenatal development, have been found in patients with ASD (Fatemi, et al., 2005). In addition, it has been proposed that the RELN gene variant, which is responsible for decreasing reelin gene expression, might result in a susceptibility to ASD (Acosta & Pearl, 2003; Persico & Bourgeron, 2006). Third, the SHANK3 gene, which is responsible for encoding scaffolding proteins that play a crucial role in maintaining and supporting dendritic spines, have been reported to exhibit a rare mutation in individuals with ASD (Durand, et al., 2007). In sum, these three lines of evidence suggest that alterations in specific genes within ASD can cause disruptions in

synaptogenesis, cell migration and dendritic morphology. This in turn can have a tremendous impact on alterations in brain connectivity in ASD.

Indeed, individuals with ASD show evidence for alterations in structural and functional brain connectivity. For example, histological studies on post-mortem brain tissue have provided evidence for abnormalities in cortical cell organization (Bailey, et al., 1998; Casanova, 2007; Hutsler, Love, & Zhang, 2007). In addition to post-mortem studies, structural MRI studies have found evidence for neuroanatomical disturbances in grey and white matter and these findings have led to suggestions that ASD is characterized by increases in short- range connections and decreases in long-range connections (Hardan, Muddasani, Vemulapalli, Keshavan, & Minshew, 2006; Herbert, et al., 2004). Similarly, functional MRI studies have also provided evidence for abnormal patterns of functional connectivity within ASD. Specifically, higher association brain areas that are involved in language and social cognition show weaker functional connectivity between structures in ASD relative to controls (Just, Cherkassky, Keller, Kana, & Minshew, 2007; Kana, Keller, Cherkassky, Minshew, & Just, 2006; Kleinhans, et al., 2008; Koshino, et al., 2008; Villalobos, Mizuno, Dahl, Kemmotsu, & Muller, 2005; Welchew, et al., 2005; Wicker, et al., 2008). This pattern of weaker functional connectivity within ASD relative to controls might suggest less synchrony between brain structures and could yield a more disconnected system (Courchesne & Pierce, 2005). These patterns of findings might relate to specific mutations or variations in the genes that facilitate cell migration, which occurs during the first 6 months of gestation (Piven, et al., 1990). In addition to reports of weaker functional connectivity in ASD, a few studies have also noted areas of tighter functional connectivity in individuals with ASD

relative to controls (Mizuno, Villalobos, Davies, Dahl, & Muller, 2006; Turner, Frost, Linsenbardt, McIlroy, & Muller, 2006). This might be a result of abnormalities within genes that are involved in the process of synaptogenesis (Varoqueaux, et al., 2006). It is important to note that all of the studies that reported abnormal functional connectivity have been carried out in adults, and none have examined functional connectivity in a sample of younger individuals.

Adolescence is a period when dynamic changes take place. It is during this time that interactions with peers begin to be more complex, and social cognition becomes much more important in adolescence as transitions towards a larger social arena are taking place. In addition, neuroanatomical studies have also provided evidence for changes that take place in the brain during the period of adolescence (Giedd, 2008; Sowell, et al., 2003).

The aim of the dissertation was to study functional connectivity using two complementary methods in adolescents with ASD and to examine how functional connectivity related to specific impairments in ASD. Since adolescence is a period when individuals are exposed to a multitude of social situations, it is of interest to study how functional connectivity changes within the context of a social task in individuals with ASD.

To this end, Chapter II examines functional connectivity between the amygdala and the superior temporal sulcus as well as connectivity between the amygdala and the inferior frontal gyri in a task involving emotional faces. We employed a technique termed psychophysiological interaction (PPI) (Friston, et al., 1997; Gitelman, Penny, Ashburner, & Friston, 2003). PPI goes beyond traditional functional connectivity analyses, which

rely on correlations between areas of the brain without regard to task condition. PPI provides information about how two brain structures may work in concert during various task conditions. PPI uses a general linear model to examine the interaction of task condition (known as the psychological variable), with the hemodynamic response of a brain seed region (known as the physiological variable). The dependent variable is the hemodynamic response of the brain. Three regressors are entered into the regression equation: Our first regressor, the physiological variable, was obtained by extracting and averaging the time courses of an 8 mm sphere around a seed voxel. Our seed voxel was placed in the amygdala since this structure is known to be involved in socio-emotional processing, and it shows alterations in ASD. This regressor accounts for the simple effect of the seed regions hemodynamic response. Our second regressor, the psychological variable, was the task condition and corresponded to emotional (fearful, happy and sad) faces relative to baseline. This regressor accounts for the simple effect of task condition. Our third regressor was the interaction between the physiological and the psychological variables. This regressor accounts for how strength of connectivity between the seed region and the rest of the brain may vary by task condition. In functional connectivity techniques, the assumption is that if two brain regions are working in tandem with one another during a cognitive process, then there would be a strong correlation in their time courses, signifying a greater connectivity or coupling between brain structures. If there is a low degree of synchrony in time courses, connectivity is said to be weak; conversely, a high degree of synchrony is considered strong connectivity. Connectivity may also be strongly positive, signifying a positive correlation between brain regions, or strongly negative, signifying a large negative correlation. This technique enables us to identify

patterns of connectivity in adolescents with ASD relative to controls and how connectivity relates to degree of social impairment in ASD.

Chapter III examined functional connectivity in the default network in the absence of a cognitive task in adolescents with ASD. The default network has been identified as a group of structures, which are active independent of specific external stimuli or explicit cognitive tasks. This intrinsic or resting-state activation can be measured by placing a seed within the default network and measuring correlations over time with other regions in the brain. In this study, we used a seed within the posterior cingulate cortex (PCC) to examine how other brain regions in the default network correlated with activity within the PCC seed. This provided information about functional connectivity within the default network. In addition, it enabled us to examine patterns of connectivity in adolescents with ASD relative to controls and to document how measures of symptom severity and adaptive behavior related to functional connectivity within the default network.

Together, these studies aim to elucidate the functional connectivity pattern in adolescents with ASD. A related goal is to document how alterations of functional connectivity seen in ASD map onto specific clinical impairments, such as social function and adaptive behavior. Examining functional connectivity in ASD can serve as an important and complementary technique to existing reports of genetic abnormalities that result in disturbances in neural development. More directly, mapping out specific changes in functional connectivity can provide a basis of how genetic mutations can disrupt brain connectivity in ASD.

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CHAPTER II

DISTURBANCES OF FUNCTIONAL CONNECTIVITY BETWEEN THE AMYGDALA AND CORTICAL NETWORKS THAT UNDERLIE EMOTIONAL FACE PROCESSING IN AUTISM SPECTRUM DISORDERS

ABSTRACT

Objective: Individuals with Autism Spectrum Disorders (ASD) have widespread deficits in the social domain. The inability to extract social information from emotional faces can make social interactions difficult. Previous neuroimaging studies on emotional face processing in ASD have focused largely on dysfunction within separate brain areas. However, no known published study has examined neural interactions between the amygdala and cortical areas involved in emotional facial expressions in adolescents with ASD. In the present study, we sought to explore functional connectivity between the amygdala and the superior temporal sulcus (STS) as well as between the amygdala and inferior frontal gyri while the participants viewed emotional faces. To ensure that attention did not differ between groups, emotional faces were presented briefly and participants' attention was engaged by way of a gender identification task as they viewed each emotional face. In addition, the task was designed to elicit robust amygdala activation in both groups. **Methods:** 27 participants (13 ASD and 14 controls) completed a functional MRI (fMRI) study while performing a gender identification task on a set of emotional (fearful, happy and sad) and neutral faces. In addition, accuracy and mean reaction times from a post fMRI behavioral emotional recognition task were collected.

Results: There were no differences in accuracy or mean reaction time between the ASD and control groups in identifying the gender of the emotional faces, signifying that both groups were attending to the emotional face stimuli. Additionally, both groups showed robust amygdala activation to emotional (fearful, happy and sad) and neutral faces versus baseline. When we examined functional connectivity, adolescents with ASD relative to controls, showed less positive connectivity between the amygdala and the right STS to emotional faces versus baseline. In addition, adolescents with ASD showed less positive connectivity between the amygdala and the inferior frontal gyrus to sad faces versus baseline. An analysis of severity of symptoms within the ASD group revealed that the degree of social impairments was related to altered connectivity between the amygdala and cortical (temporal and frontal) regions. **Conclusions:** Adolescents with ASD exhibited less positive functional connectivity between the amygdala and cortical areas involved in emotional face processing and empathy. These disturbances provide evidence for alterations in connectivity that could underlie deficits in social cognition.

INTRODUCTION

Autism Spectrum Disorders (ASD) are a set of neurodevelopmental conditions characterized by deficits in social and communicative functioning, in the presence of marked repetitive and restricted interests (APA, 1994). One of the most debilitating components of ASD is the impairment in social functioning. It has been suggested that social impairments, such as the lack of eye gaze and joint attention, both important developmental milestones in typically developing children, can eventually jeopardize the development of language communication and social engagement (Dawson, Webb, & McPartland, 2005; Schultz, et al., 2003).

In typically developing individuals, successful navigation of the social world hinges upon the ability to read emotional expressions on faces (Elgar & Campbell, 2001). The emotional content of a face immediately signals a person's internal state (Ekman, 1993), allowing others the opportunity to read the signals and either continue with a desired behavior (e.g., in response to a happy expression) or adjust behavior (e.g., in response to an expression of disgust). This information guides how we behave and helps us to navigate the social world. In ASD, a large number of studies have noted various face processing related difficulties. These difficulties include deficits in emotional recognition (Humphreys, Minshew, Leonard, & Behrmann, 2007) and reduced attention to the eye region (Dalton, et al., 2005; Klin, Jones, Schultz, Volkmar, & Cohen, 2002). It has been suggested that face-processing difficulties may underlie the core symptom of social impairment (Klin, et al., 1999; Schultz, et al., 2000).

Seminal studies on emotional processing in human patients with amygdala damage (Adolphs, Baron-Cohen, & Tranel, 2002; Adolphs, Damasio, Tranel, & Damasio,

1996) have highlighted the crucial role that the amygdala plays in the identification and interpretation of emotional stimuli (Haxby, Hoffman, & Gobbini, 2002). Additional work by Anderson and Phelps (2000) reported profound impairment in the ability to evaluate facial expressions in patients with amygdala lesions (Anderson & Phelps, 2000). Consequently, neuroimaging studies have focused on the role of the amygdala in emotional face processing (Breiter, et al., 1996; Morris, et al., 1996; Sato, Yoshikawa, Kochiyama, & Matsumura, 2004; Vuilleumier, Schwartz, Clarke, Husain, & Driver, 2002; Whalen, et al., 1998; P. Wright & Liu, 2006). Since ASD is often seen as a disorder of social-emotional function (Bachevalier & Loveland, 2006; Nacewicz, et al., 2006), fMRI studies in individuals with ASD have focused on studying the amygdala in face processing and have documented disturbances within this structure (Ashwin, Baron-Cohen, Wheelwright, O'Riordan, & Bullmore, 2007; Critchley, et al., 2000; Grelotti, et al., 2005; Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2007). However, since effective social functioning engages a network of structures including the amygdala (Haxby, et al., 2002), it is also important to examine how this network is altered in ASD.

There have only been five studies that have investigated functional connectivity between the amygdala and cortical areas involved in emotional processing in ASD (Hadjikhani, et al., 2007; Kleinhans, et al., 2008; Monk, et al., *under review*; Welchew, et al., 2005; Wicker, et al., 2008). However, with the exception of the Monk et al., (*under review*) study, none of these studies utilized the amygdala as their main seed region. In addition, none of these studies examined functional connectivity in a sample of adolescents. There have been an increasing number of studies focusing on functional connectivity in other neural networks implicated in ASD (Kana, Keller, Cherkassky,

Minshew, & Just, 2006; Kleinhans, et al., 2008; Koshino, et al., 2008; Mizuno, Villalobos, Davies, Dahl, & Muller, 2006; Turner, Frost, Linsenbardt, McIlroy, & Muller, 2006; Villalobos, Mizuno, Dahl, Kemmotsu, & Muller, 2005). Beyond the amygdala, the network of regions involved in face processing includes regions in the temporal and frontal cortices. In fact, a neuroimaging study found that the blood-oxygen-level-dependent (BOLD) activation in several brain areas were highly correlated with each another during face processing (Hadjikhani, et al., 2007). Evidence from histological studies in rhesus monkeys suggest that emotional information is relayed from the temporal areas to the amygdala and back to cortical areas such as the orbital frontal regions (Hoistad & Barbas, 2008; McDonald, 1998).

The superior temporal sulcus (STS), which divides the superior temporal and middle temporal gyri in the temporal lobe, is a key region involved in face processing (Engell & Haxby, 2007; Puce, Allison, Bentin, Gore, & McCarthy, 1998). Electrophysiological studies revealed that the cells of the STS in monkeys showed an increase in firing rate when faces were presented (Perrett, et al., 1985). In humans, studies have shown that the STS plays a key role in responding to faces (Haxby, Hoffman, & Gobbini, 2000; Haxby, et al., 2002; Materna, Dicke, & Thier, 2008). Specifically, the STS has been identified as a region that is involved in processing the dynamic nature of emotional facial expressions (Haxby, et al., 2000). In the fMRI literature, numerous studies have noted increases in activation of the STS region when emotional face stimuli are presented (Hein & Knight, 2008; Materna, et al., 2008; Puce & Perrett, 2003). In ASD, some authors have found evidence for neuroanatomical (Boddaert, et al., 2004; Levitt, et al., 2003) and functional abnormalities (Pelphrey &

Carter, 2008) within the STS. One subregion of the STS (Hein & Knight, 2008) may be particularly relevant for face processing: studies have provided evidence that emotional face processing recruits an area of the STS in the middle temporal gyrus (Adolphs, et al., 1996; Materna, et al., 2008) that is slightly more posterior (Hein & Knight, 2008; Materna, et al., 2008) and often lateralized to the right hemisphere (Adolphs, et al., 1996). Apart from being an area that is heavily involved in face processing, strong connections between the amygdala and the medial temporal poles relative to the lateral temporal poles have been reported (Hoistad & Barbas, 2008; McDonald, 1998) and primate studies have recently discovered that when face-selective cells within the temporal lobe were stimulated in monkeys, these stimulations induced activation in subcortical structures such as the amygdala (Moeller, Freiwald, & Tsao, 2008).

The mirror neuron system (MNS) has been proposed as a neural mechanism that underlies aspects of social cognition by forming the scaffold from which individuals comprehend other's behavior, intentions and feelings (Pfeifer, Iacoboni, Mazziotta, & Dapretto, 2008). The MNS was first introduced when researchers found evidence for a group of neurons in region F5 of the monkey cortex that was selectively active during imitation tasks (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996). In humans, this key MNS region has been identified in neuroimaging studies as the inferior frontal gyrus (IFG) (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003; Dapretto, et al., 2006; Decety & Moriguchi, 2007; Leslie, Johnson-Frey, & Grafton, 2004; Schulte-Ruther, Markowitsch, Fink, & Piefke, 2007; Shamay-Tsoory, Aharon-Peretz, & Perry, 2008).

It has been suggested that social abilities which are particularly impaired in ASD, such as imitation, joint attention and theory of mind are subserved by the MNS and are

particularly impaired in ASD (Villalobos, et al., 2005; Williams, Whiten, Suddendorf, & Perrett, 2001). Indeed, recent findings indicate that hypoactivation within the MNS area of the IFG during imitation related to greater social impairment in children with ASD (Dapretto, et al., 2006). In addition, studies have found that activation in the IFG correlates with the ability to understand another's emotional state (Hooker, Verosky, Germine, Knight, & D'Esposito, 2008; Pfeifer, et al., 2008; Saarela, et al., 2007). The ability to empathize is crucial to successful social functioning and there is evidence that individuals with ASD are less adept at empathizing (Baron-Cohen & Wheelwright, 2004; Dziobek, et al., 2008).

Neuroimaging studies of empathy in typically developing individuals have noted that there is increased IFG activation when participants viewed different facial expressions (Nomi, et al., 2008). Specifically, studies have identified this region when participants listened to sad stories (Decety & Chaminade, 2003), performed tasks which required them to decipher what someone is thinking and feeling (Farrow, et al., 2001; Hynes, Baird, & Grafton, 2006), and when presented with frowning/sad and happy expressions (Kim, et al., 2005; Lee, Dolan, & Critchley, 2008; Wild, Erb, & Bartels, 2001). This region of the frontal cortex is part of a complex neural network with dense connections to limbic areas such as the amygdala (Barbas, 2007; Rempel-Clower, 2007). In addition, there has been evidence that the amygdala is also involved in the neural substrates of empathy (Carr, et al., 2003; Stone, Baron-Cohen, Calder, Keane, & Young, 2003) and that the IFG modulates the activity in the amygdala (Pfeifer, et al., 2008). Indeed, an fMRI study in typically developing individuals reported that the activity in the amygdala correlated positively with activity in the inferior frontal gyrus and that these

two regions exhibited positive connectivity during an emotional face task (Iidaka, et al., 2001). Therefore, dysfunction in connectivity between these areas can have a profound impact on processing the emotional content on faces (Blair, Morris, Frith, Perrett, & Dolan, 1999).

Goals of this study

In the present study, we explored the relationship between neural regions underlying emotional face processing in adolescents with ASD relative to controls while they made gender identification judgments to a set of emotional faces. The fMRI paradigm was uniquely designed to elicit robust amygdala activation in both groups while controlling for attention via short face presentations (250 ms). Typically developing children show robust amygdala activation to emotional faces (Guyer, et al., 2008; Monk, et al., 2003). In ASD, differences across tasks may account for mixed findings in amygdala activation. Although there have been reports of hyperactivation (Dalton, et al., 2005) as well as hypoactivation in the amygdala (Ashwin, et al., 2007; Critchley, et al., 2000; Grelotti, et al., 2005; Hadjikhani, et al., 2007; Pelphrey, Morris, McCarthy, & Labar, 2007), some have suggested that these discrepancies are due to attention differences in both groups (Dalton, et al., 2005; Monk, et al., *under review*). Indeed, when attention was considered, individuals with ASD elicited robust amygdala activation (Dalton, et al., 2005). In addition, a study by Pierce and colleagues (2004) found no difference in amygdala activation in both groups when making gender identification judgments (Pierce, Haist, Sedaghat, & Courchesne, 2004).

In order to examine these network differences, we adopted a method termed psychophysiological interaction analysis (PPI) (Friston, et al., 1997; Gitelman, Penny,

Ashburner, & Friston, 2003). This type of analysis enabled us to compare the connectivity/ coupling between a seed region (amygdala) and other areas of the brain implicated in face processing during different task conditions. Recent studies have successfully employed this technique to examine how other brain areas involved in emotional face processing interact with the amygdala (Foland, et al., 2008; Iidaka, et al., 2001; Passamonti, et al., 2008). Because some authors have suggested that individuals with ASD might orient away from social stimuli (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998), the gender identification task ensured that the participants were attending to faces. In addition, the brief facial presentations made it unlikely for participants to make saccades away from the stimulus and for differences in facial gaze patterns to occur (Clark, Winkielman, & McIntosh, 2008).

First we hypothesized that, adolescents with ASD, relative to controls, would show less positive coupling between the amygdala and the right middle temporal gyrus to emotional faces versus baseline. This was based on 5 lines of evidence. First, studies have identified the right middle temporal gyrus as a key region in emotional face processing (Haxby, et al., 2000). Second, there have been reports of hypoactivation within this area in individuals with ASD (Hadjikhani, et al., 2007; Pierce, Muller, Ambrose, Allen, & Courchesne, 2001). Fourth, the amygdala and temporal areas are robustly interconnected (Hoistad & Barbas, 2008; McDonald, 1998). Fifth, previous findings in our lab found less positive connectivity between the amygdala and the temporal lobe in adults with ASD when performing an attention cueing task with emotional faces (Monk, et al., under review).

Second, we hypothesized that adolescents with ASD, relative to controls, would

show less positive coupling between the amygdala and the inferior frontal gyri (IFG) to sad and happy faces versus baseline. This hypothesis was specific to sad and happy faces since previous studies in empathy have consistently employed sad and happy facial expressions to elicit feelings of empathy. The second hypothesis was based on 3 lines of evidence. First, the IFG, a component of the MNS is recruited during tasks involving empathy (Shamay-Tsoory, et al., 2008). This is of special interest in ASD since many studies have reported deficits in empathizing abilities (Baron-Cohen & Wheelwright, 2004; Dziobek, et al., 2008). Second, the amygdala has been reported to have connections to the inferior frontal gyrus and both these areas play a role in evoking feelings of empathy to sad and happy faces (Decety & Chaminade, 2003). Third, previous findings in our lab noted that adults with ASD show less positive coupling between the amygdala and IFG to sad versus neutral faces relative to typically developed adults (Monk, et al., *under review*).

Third, we hypothesized that greater social impairment, as reflected by higher severity scores in the social domains of diagnostic measures would relate to less positive coupling between the amygdala and areas within the right middle temporal gyrus as well as within the inferior frontal gyrus. This was based on 2 lines of evidence. First, a prior study reported that individuals with ASD who had greater social impairment, often displayed weaker functional connectivity between brain structures (Kleinhans, et al., 2008). Second, reports of hypoactivation in the right middle temporal gyrus (Wang, Lee, Sigman, & Dapretto, 2007) as well as the inferior frontal gyrus (Dapretto, et al., 2006) were found to be associated with greater social impairment.

METHODS

Participants

Sixteen adolescents with ASD and fourteen controls participated in the study. In the ASD group, one adolescent was excluded due to excessive head movement and two did not complete the scan due to nervousness/anxiety. The final set included 13 adolescents with ASD with an age range between 13 to 17 years old (12 males, 1 female) and 14 controls with an age range between 13 to 18 years old (13 males, 1 female). Of the 13 adolescents with ASD, 4 were diagnosed with autism, 1 with Asperger's syndrome (AS) and 8 with pervasive developmental disorder not otherwise specified (PDD-NOS). All ASD participants were diagnosed 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 confirmed by clinical consensus. Seven of the 13 ASD participants were on psychotropic medication (2 were on selective serotonin reuptake inhibitors, 5 were on stimulants, 3 were on neuroleptics and 1 was on atomoxetine). Post-hoc analyses were carried out to ascertain if the medications contributed to group differences (refer to Results). Verbal and non-verbal cognitive functioning was obtained by administering the Peabody Picture Vocabulary Test (PPVT) (Dunn & Dunn, 1997) and the Ravens Progressive Matrices (Raven, 1960) respectively. There were no significant group differences in age, verbal cognitive functioning, nonverbal cognitive functioning, gender, and handedness (refer to Table 2.1). All adolescents with ASD were recruited through the University of Michigan Autism and Communication Disorders Center (UMACC) and controls were recruited through advertisements and posted flyers.

Procedures

The University of Michigan Institutional Review Board approved all procedures. Interested participants were screened for eligibility and a brief description of the study, procedures and payment for participation was given to parents of all participants during an initial phone call. The controls were screened through a phone interview to exclude for adolescents on psychotropic medications or who had a history of mental disorders, surgeries or wore braces. Exclusion criteria for the ASD group were as follows: IQ < 85, or presence of a co-occurring neurological disorder or history of surgeries, or if the participants wore braces. The research study comprised of two visits. During visit 1, parents of the participants signed consent forms and filled in self-report questionnaires. In addition, parents of controls completed both the Social Communication Questionnaire (SCQ) (Rutter, et al., 2003) as well as the Social Responsiveness Scale (SRS) (Constantino, et al., 2003). These questionnaires enabled us to gain an index for the level of social functioning within the control group and allowed us to exclude for participants who scored within the ASD range on these social and communication measures. During visit 1, participants completed questionnaires and did a practice version of the fMRI task in a mock scanner to enable acclimatization to the scanner conditions. During visit 2, participants were scanned at the University of Michigan's fMRI lab. Screening forms were completed prior to entering the MRI scanner. The protocol began with the T1 overlay, followed by an fMRI task involving emotional faces, a high-resolution structural image and finally a resting connectivity task. After completion of the scan, participants completed behavioral tasks on a laptop in a separate testing room.

Experimental Paradigm

During image acquisition, participants performed gender identification judgments on a set of emotional and neutral faces. Emotional and neutral faces were selected from the NimStim Face Stimulus Set (Tottenham, et al., *in press*) (www.macbrain.org). Fearful, happy, neutral and sad faces were presented. There were 30 trials of each emotion across 2 functional runs. Trials were presented in a different randomized order for each subject.

Each trial began with a fixation cross that was displayed in the center of the screen for 500 ms, followed by a face that was displayed for 250 ms. A blank screen then replaced the face for 1500 ms. During this period, participants pressed the thumb button if they saw a male face and the index finger button if they saw a female face. Following this, an inter trial interval (ITI) that varied between 0 ms to 6000 ms (at intervals of 2000ms) was included between each trial to allow hemodynamic responses to return to baseline levels between stimulus presentations (refer to Figure 2.1). There were a total of 120 trials across the two functional runs and each run lasted for approximately six minutes. We used E-prime (Psychological Software Tools, Pittsburgh, PA) to control stimulus presentations and to record responses.

Participants were instructed to respond as quickly and as accurately as possible during the task. Prior to the MRI scan, participants completed a practice session in a mock scanner to ensure that they were comfortable with the task and testing conditions.

Data Acquisition

MRI images of the brain were acquired with a long bore 3 Tesla GE Signa operating on the 12.0 platform at the University of Michigan's fMRI lab. A GE quad head

coil was used. All participants made responses with a button box that was linked to an IFIS system (MRI Devices, Inc., Milwaukee, WI) and attached to their right hand. In addition, participants wore goggles with built-in mirrors (VisuaStim XGA, Resonance Technologies) in order to view the projected stimuli inside the scanner. For those with corrected vision, lenses were fitted into the goggles prior to scanning. To reduce noise levels associated with the MRI scan, participants wore earplugs throughout the time that they were in the scanner. For the structural images, a 3D T1 axial overlay consisting of 124 slices of 1.4 mm thickness per slice (TR=8.9, TE=1.8, flip angle=15°, FOV=26 cm; matrix=256 x160) and a high resolution sagittal SPGR image consisting of 110 slices of 1.4mm thickness per slice (flip angle=15°, FOV=26 cm) were acquired. For the functional images, T2*-weighted BOLD images were collected using a reverse spiral sequence (Glover & Law, 2001). The BOLD images were made up of 40 adjacent 3 mm axial slices (TR=2000 ms, TE=30 ms, flip angle=90°, FOV=22 cm; matrix=64x64). The slices were made adjacent to each other and parallel to the AC-PC line, to ensure that movement-related post-processing algorithms were performed optimally. The images were then reconstructed to maximize magnetic field homogeneity and ensure that the functional images were corrected for misalignment to the structural data.

Functional MRI Data Analysis

The data was passed through a series of initial preprocessing steps at the fMRI lab. First, the skull was removed using FSL (http://www.fmrib.ox.ac.uk/fsl). The scalping aids the normalization process and was carried out using the Brain Extraction Tool in FSL. Second, large spikes in the k-space data were filtered out. Third, while the data were reconstructed into images, a field map correction was carried out. Fourth, the

reconstructed images were then corrected for difference in acquisition time for each slice. Following the slice time correction using local sinc interpolation (Oppenheim, Schafer, & Buck, 1999), the images were realigned using McFlirt in FSL (Jenkinson, Bannister, Brady, & Smith, 2002). The images were then transferred from the fMRI lab server to local servers. The functional images were first examined to exclude cases with head motion greater than 3 mm in any six directions. Using SPM5 (www.fil.ion.ucl.ac.uk/spm), T1 GRE images were co-registered to the 3D SPGR volume in order to map the functional images into a standardized anatomical space. The 3D SPGR volume was then inhomogeneity-corrected and normalized using a 8 mm full width at half maximum (FWHM) Gaussian kernel to the SPM5 T1 template (MNI space).

Statistical processing of the functional data was carried out using the SPM5 software. All incorrect trials and trials without responses on the fMRI task were excluded from the analysis. General Linear Model (GLM) and random effects analyses were utilized to assess within- and between-group effects. For each participant, a statistical image for each contrast at each voxel was generated. The contrast maps generated for each participant was then put into the GLM to test population-level hypotheses.

The primary purpose of this study was to examine functional connectivity between the amygdala and 1) inferior frontal gyrus (left and right separately) and 2) right middle temporal region. A region of interest (ROI) approach was adopted to confirm that there was significant amygdala activation in both groups prior to examining functional connectivity. This ROI approach within the left amygdala and right amygdala is consistent with other studies (Fakra, Salgado-Pineda, Delaveau, Hariri, & Blin, 2008) and the ROIs were defined according to the WFU Pickatlas toolbox

(http://www.fmri.wfubmc.edu/) (Maldjian, Laurienti, Burdette, & Kraft, 2002). In order to control for multiple comparisons, small volume correction (SVC) was used (Worsley, et al., 1996) on the left amygdala and the right amygdala and reported the family wise error (FWE) of 0.05 or less, cluster size $k \geq 10$ voxels unless otherwise specified, within each SVC-corrected region.

Functional Connectivity Analysis

In order to explore functional connectivity between the amygdala and the temporal and frontal cortices, we carried out a psychophysiological interaction (PPI) analysis (Friston, et al., 1997; Gitelman, et al., 2003). This type of analysis allowed us to study the patterns of relationships between two areas of the brain during different task conditions. This method allowed us to compare the strength of the functional relationship between areas of the brain during different parts of the task. PPI is aptly named, as it examines the interaction effect of the psychological variable (task condition), and the physiological variable (brain seed region BOLD response). In a PPI regression equation, the dependent variable is the hemodynamic response of the brain, while the predictors are the interaction of the task condition by the hemodynamic response of the brain seed region, and the lower order terms. In our study, the physiological variable corresponded to the average time course extracted from an 8 mm diameter sphere around a voxel in the amygdala (seed region). The seed was determined at the group level and was the same for each participant. The psychological variable was the trial/contrast of interest (e.g. fearful versus baseline). The PPI analysis was conducted using SPM5 package.

In order to examine our a priori hypothesis of connectivity in a specific region of the right middle temporal gyrus and the inferior frontal gyrus/orbital region, we used the

WFU Pickatlas toolbox (Maldjian, et al., 2002) to select our ROIs for each of these areas. First, to create a mask for the right middle temporal gyrus, we used the advanced function in WFU Pickatlas to select the portion of the right middle temporal gyrus that was within BA37 by intersecting the two regions. To ensure that the entire cluster was captured, we dilated each region separately, prior to intersecting. In addition, to ensure that only regions within the temporal lobe were included in our mask, we intersected our intermediate mask with the temporal lobe to create the final mask that was used for the fMRI analysis of the right middle temporal gyrus. Second, for the inferior frontal gyri, we used the Inf_Frontal_Orbital_R and Inf_Frontal_Orbital_L separately within the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer, et al., 2002) that was found in the WFU Pickatlas toolbox. In order to control for multiple comparisons, we used SVC (Worsley, et al., 1996) on the right middle temporal gyrus mask, the left inferior frontal gyrus mask and the right inferior frontal gyrus mask. In this present chapter, we reported the FWE of 0.05 or less, cluster size $k \geq 10$ voxels, unless otherwise specified, within each SVC corrected region.

In order to examine the relationship between severity of social symptoms and strength of functional connectivity within the ASD group, we entered social severity scores obtained from several measures as covariates in the multiple regression analysis in SPM5. This enabled us to examine positive and negative associations within the ROIs that were used in the PPI analysis. The measures that we used were the social components from the ADI-R and the ADOS, as well as the SRS. The ADI-R score was computed to obtain both "ever" (includes impairment seen at any point in the individual's life) and "current" (which only codes impairment seen 3 months prior to clinical

assessment) codes. This resulted in a total of 4 social measures that were used. In order to control for multiple comparisons within analyses, we performed a Bonferroni correction to account for the 4 measures that were used. This resulted in a threshold of p=0.0125. In addition, only cluster sizes of $k \geq 10$ are reported.

Behavioral Data Analysis

fMRI task (face task performed during fMRI acquisition):

Overall task accuracy, as well as accuracy and mean reaction time to each type of emotional face (fearful, happy, neutral and sad) was obtained. The gender identification judgments made during this emotional face task, allowed us to ensure that the participants were indeed attending to the faces presented. Performance and reaction time data were collected and analyzed both at the individual and group level.

Post fMRI task (emotional recognition task performed on a laptop):

Following the completion of the face task in the fMRI scanner, participants were also given an emotional recognition behavioral task. The task was designed to assess performance in emotion recognition. The task was administered immediately after participants came out of the fMRI scanner and was completed on a laptop in a separate testing room. The face set comprised of the same fearful, happy, neutral and sad faces that were shown in the fMRI face task. There were a total of 120 trials (with equal representation of each emotional face). The faces were presented in a different randomized order for each participant.

Trials began with a fixation cross in the middle of the screen for 500 ms, followed by the face for 250 ms and a screen which displayed the instructions, prompting the participant to respond accordingly: Press 1 if the face is happy, press 2 if the face is

neutral, press 3 if the face is sad, and press 4 if the face is fearful. Subsequent trials were displayed only after the participant made a response. In order to give the participants the opportunity to make a response, the time limit on each trial was set at 7000 ms. We used E-prime (Psychological Software Tools, Pittsburgh, PA) to control stimulus presentations and to record responses.

Participants were asked to respond as soon as they could discern the emotion on each face. Participants completed a short practice session prior to the emotional recognition task to ensure that they understood the instructions and were comfortable with the task.

RESULTS

Behavioral results

fMRI task (face task performed during fMRI acquisition):

There were no significant differences between the ASD and control group in overall task accuracy, $t(25) = 1.35$, $p = 0.19$ and mean reaction time for correct responses $t(25) = 0.92$, $p = 0.37$. The ASD group had a mean accuracy of 95.3% and control group had a mean accuracy of 96.9%. The mean reaction time of the correct responses for the ASD group was 736.7 (±135.86) ms and the mean reaction time for the control group was 693.01 (±110.32) ms.

Mean accuracy and mean RT for correct responses across each emotion was reported (refer to Table 2.2). When making gender identifications to fearful faces, there were no significant differences between the ASD and control group in accuracy $t(25) = 1.54$, $p = 0.14$ and mean reaction time $t(25) = 1.00$, $p = 0.32$. To happy faces, there were no group differences between the ASD and control group in accuracy, $t(25) = 1.26$, $p =$

0.22 and mean reaction time $t(25) = 0.90$, $p = 0.38$. To sad faces there were no group differences between the ASD and control group in accuracy, $t(25) = 0.76$, $p = 0.45$ and mean reaction time $t(25) = 1.11$, $p = 0.32$. Finally, to neutral faces, there were no group differences between the ASD and control group in accuracy $t(25) = 0.59$, $p = 0.56$ and mean reaction time $t(25) = 0.67$, $p = 0.51$.

Post fMRI task (emotional recognition task performed on a laptop):

There were no significant differences between the ASD and control group in overall task accuracy, $t(25) = 6.08$, $p = 0.548$ and mean reaction time for correct responses $t(25) = 0.85$, $p = 0.40$. The ASD group had a mean accuracy of 89.1% and ranged from 68.3% to 95.8%. The control group had a mean accuracy of 90.7% and ranged from 74.6% to 96.7%. The mean reaction time of the correct responses for the ASD group was 1200.08 (±247.27) ms and the mean reaction time for the control group was 1131.57 (±167.49) ms. Because this task was designed to allow participants the opportunity to label the emotion on each face, more emphasis was placed on determining accuracy of each group across emotion rather than examining reaction time differences.

Mean accuracy and mean RT for correct responses across each emotion was reported (refer to Table 2.3). When labeling fearful faces, there were no significant differences between the ASD and control group in accuracy, $t(25) = 1.04$, $p = 0.31$ and mean reaction time $t(25) = 0.37$, $p = 0.72$. To happy faces, there were no group differences between the ASD and control group in accuracy, $t(25) = 0.81$, $p = 0.43$ and mean reaction time $t(25) = 0.05$, $p = 0.96$. To sad faces there were no group differences between the ASD and control group in accuracy $t(25) = 0.14$, $p = 0.89$ and mean reaction time $t(25) = 1.56$, $p = 0.13$. Finally, to neutral faces, there were no group differences

between the ASD and control group in accuracy $t(25) = 0.45$, $p = 0.66$ and mean reaction time $t(25) = 1.78$, $p = 0.09$.

fMRI *activation in the amygdala*

The main purpose of this study was to explore functional connectivity between the amygdala and regions in the temporal and frontal cortices in adolescents with ASD relative to controls. In order to examine our hypothesis, we conducted an ROI analysis on the amygdala to confirm that reliable amygdala activation was observed in both groups. At $p = 0.05$, small volume correction (SVC) on the left and right amygdala revealed robust bilateral amygdala activation in both groups when comparing each of the faces (fearful, happy, sad and neutral) relative to baseline (refer to Figure 2.2). We did not find significant group differences in amygdala activation between the ASD and control group in these analyses. In addition, emotional faces relative to neutral comparisons did not yield amygdala activation (refer to Table 2.4). When we explored whole brain activation outside areas of the amygdala, we found robust activation throughout the brain and multiple regions showed group differences (refer to Appendix Table 1).

Functional connectivity between the amygdala and the temporal and frontal corticies

To evaluate functional connectivity between the amygdala and the temporal and frontal cortices, we carried out PPI analysis on contrasts that showed significant amygdala activation in both groups (ASD and control groups combined) (refer to Table 2.4). The left and right amygdala seeds that we utilized for the PPI corresponded to voxels in the amygdala that showed peak activation within each contrast of interest (fearful vs. baseline: -20 -08 -14 and 18 -8 -14; happy vs. baseline: -22 -8 -10; sad vs. baseline: -20 -2 -14 and 24 -8 -10 and neutral vs. baseline: -22 -2 -14 and 24 -04 -20).

To evaluate our first hypothesis that adolescents with ASD would show less positive connectivity/coupling between the amygdala and the right middle temporal gyrus relative to controls, we used the PPI analysis to examine group differences in connectivity between the amygdala and the right middle temporal gyrus when participants were viewing emotional faces. Consistent with the hypothesis, controls showed greater positive coupling between the left amygdala and the right middle temporal region relative to the ASD group in the contrast of fearful vs. baseline trials, $t(25) = 5.02$, $p = 0.004$ (SVC corrected), xyz coordinates = 46 -48 -8 (refer to Figure 2.3). In sad vs. baseline trials, controls again showed greater positive coupling between the right amygdala and the right middle temporal region relative to the ASD group, $t(25) = 3.72$, $p = 0.042$ (SVC corrected), xyz coordinates = 44 -72 6 (refer to Figure 2.4). In happy vs. baseline trials, controls showed a similar trend that did not reach our corrected threshold for greater positive coupling between the left amygdala and the right middle temporal region, relative to the ASD group, $t(25) = 3.46$, $p = 0.001$ (uncorrected), xyz coordinates = 48 -30 -16 (refer to Figure 2.5). In the neutral vs. baseline trials, we did not find group differences in connectivity between the amygdala and the right middle temporal region.

To evaluate our second hypothesis that adolescents with ASD relative to controls would show less positive coupling between the amygdala and the inferior frontal gyri in sad vs. baseline and happy vs. baseline conditions, we used PPI analysis to examine group differences in connectivity between the amygdala and both the left and right inferior frontal gyrus when participants were viewing emotional faces. Consistent with the hypothesis, controls relative to the ASD group showed greater positive coupling

between the left amygdala and the right inferior frontal gyrus, $t(25) = 3.88$, $p = 0.042$ (SVC corrected), xyz coordinates = 40 32 -16 (refer to Figure 2.6). There was a similar trend that did not reach our corrected threshold, between the right amygdala and the left inferior frontal gyrus $t(25) = 3.50$, $p = 0.001$ (uncorrected), xyz coordinates = -40 34 -6 (refer to Figure 2.7). These findings within the left and right inferior frontal gyrus were specific to the sad vs. baseline condition only. In the happy vs. baseline condition, we did not see significant group differences when examining the connectivity between the amygdala and the inferior frontal gyri.

fMRI activation in the right middle temporal gyrus and inferior frontal gyrus

In order to better characterize whether group differences in BOLD responses within each individual ROI for the right middle temporal gyrus and the inferior frontal gyrus contributed to group differences in the functional connectivity (PPI) analysis, we examined brain activation within each of the ROIs, in conditions where group differences in functional connectivity were found.

Right middle temporal gyrus:

In the fearful vs. baseline condition, the ASD group alone showed activation in the right middle temporal gyrus, $t(25) = 5.60$, cluster size $k = 634$, $p = 0.001$ (SVC corrected), xyz coordinates= 56 -68 6. The control group alone showed activation in the right middle temporal gyrus, $t(25) = 9.44$, cluster size $k = 808$, $p < 0.001$ (SVC corrected), xyz coordinates= 56 -68 6. Finally, there were no significant group differences between the ASD and control groups within the right middle temporal gyrus that surpassed the SVC threshold.

In the sad vs. baseline condition, the ASD group alone showed activation in the right middle temporal gyrus, $t(25) = 8.95$, cluster size $k = 930$, $p < 0.001$ (SVC corrected), xyz coordinates= 56 -68 4. The control group alone showed activation in the right middle temporal gyrus, $t(25) = 13.25$, cluster size $k = 870$, $p < 0.001$ (SVC corrected), xyz coordinates= 56 -68 4. Finally, the ASD group relative to the control group showed greater activation in the right middle temporal gyrus, $t(25) = 3.76$, cluster size $k = 264$, $p < 0.048$ (SVC corrected), xyz coordinates= 56 -68 0.

In the happy vs. baseline condition, the ASD group alone showed activation in the right middle temporal gyrus, $t(25) = 7.73$, cluster size $k = 1041$, $p < 0.001$ (SVC corrected), xyz coordinates= 52 -68 0. The control group alone showed activation in the right middle temporal gyrus, $t(25) = 10.36$, cluster size $k = 1049$, $p < 0.001$ (SVC corrected), xyz coordinates= 48 -72 -6. Finally, the ASD group relative to the control group showed greater activation in the right middle temporal gyrus, $t(25) = 4.17$, cluster size $k = 687$, $p < 0.022$ (SVC corrected), xyz coordinates= 50 -52 -6.

Inferior frontal gyrus (left and right separately):

In the sad vs. baseline condition, the ASD group alone showed activation in the left inferior frontal gyrus, $t(25) = 3.94$, cluster size $k = 467$, $p = 0.039$ (SVC corrected), xyz coordinates= -40 16 -14. The control group alone showed activation in the left inferior frontal gyrus, $t(25) = 7.15$, cluster size $k = 476$, $p < 0.001$ (SVC corrected), xyz coordinates= -36 24 -4. Finally, there were no significant group differences between the ASD and control groups within the left inferior frontal gyrus that surpassed the SVC threshold.

In the sad vs. baseline condition, the ASD group alone did not show significant

activation in the right inferior frontal gyrus that surpassed the SVC threshold. The control group alone showed activation in the right inferior frontal gyrus, $t(25) = 9.01$, cluster size $k = 765$, $p < 0.001$ (SVC corrected), xyz coordinates= 44 24 -8. Finally, there were no significant group differences between the ASD and control groups within the right inferior frontal gyrus that surpassed the SVC threshold.

Correlation between social impairments and strength of connectivity

To evaluate our hypothesis that greater social impairments would relate to less positive coupling in adolescents with ASD, we performed multiple regression with the symptom scores as a covariate in the analysis. This analysis was performed in the right middle temporal regions, the left inferior frontal gyrus and the right inferior frontal gyrus within the contrasts where group differences and notable trends were reported in the PPI analysis described above.

Right middle temporal region:

Consistent with our hypothesis, at a Bonferroni corrected threshold of $p = 0.0125$, there was a negative correlation in the contrast of happy vs. baseline between the ADI-R current score and the strength of connectivity between the amygdala and the right middle temporal regions, $t(11) = 3.77$, $p = 0.002$, xyz coordinate = 50 -56 -8 (refer to Figure 2.8). Similarly, there was a negative correlation in the contrast of fearful vs. baseline between the ADI-R ever score and the strength of connectivity between the amygdala and the right middle temporal regions, $t(11) = 3.00$, $p = 0.006$, xyz coordinate = 50 -52 -16 (refer to Figure 2.9). Finally, neither the ADOS nor the SRS yielded significant findings that surpassed the predefined threshold.

Inferior frontal gyrus:

Contrary to our hypothesis, there was a positive correlation in the contrast of sad vs. baseline between the ADI-R ever score and the strength of connectivity between the amygdala and the left inferior frontal gyrus, $t(11) = 3.01$, $p = 0.006$, xyz coordinate = -38 42 -14. Similarly, a positive correlation was noted in the contrast of sad vs. baseline between the ADI-R ever score and the strength of connectivity between the amygdala and the right inferior frontal gyrus, $t(11) = 5.70$, $p < 0.001$, xyz coordinate = 44 30 -16. Finally, neither the ADOS nor the SRS yielded significant findings that surpassed the predefined threshold.

Effects of medication

In order to examine whether medications influenced the results, adolescents with ASD who were on at least one psychotropic medication were removed from the analysis. The remaining 6 adolescents with ASD who were not on medication were compared to the controls to examine if a consistent pattern of findings in the temporal and frontal lobe prevailed. We adopted this approach following a similar fMRI study in individuals with ASD (Wang, et al., 2007).

When we evaluated the PPI results within the right middle temporal region between the remaining 6 non-medicated adolescents with ASD and the 14 controls, the control group relative to the ASD group continued to show greater positive coupling between the left amygdala and the right middle temporal region, in the fearful vs. baseline condition $t(18) = 4.71$, $p = 0.017$ (uncorrected), xyz coordinates = 46, -48 -10. In the sad vs. baseline condition, the control group relative to the ASD group continued to show greater positive coupling between the right amygdala and the right middle temporal

region $t(18) = 2.10$, $p = 0.025$ (uncorrected), xyz coordinates = 44, -72 4. In the happy vs. baseline condition, the control group relative to the ASD group continued to show greater positive coupling between the left amygdala and the right middle temporal region $t(18) = 2.95$, $p = 0.004$ (uncorrected), xyz coordinates = 52, -30 -18.

Similarly, when we evaluated the PPI results within the inferior frontal gyri between the 6 non-medicated adolescents with ASD and the 14 controls, the control group, relative to the ASD group continued to show greater positive coupling between the left amygdala and the right inferior frontal gyrus $t(18) = 3.93$, $p < 0.001$ (uncorrected), xyz coordinates = 40 32 -16, as well as the right amygdala and the left inferior frontal gyrus, $t(18) = 3.60$, $p = 0.001$(uncorrected), xyz coordinates = -40 36 -8, in the sad vs. baseline conditions.

DISCUSSION

Our findings demonstrate a pattern of weaker positive functional connectivity between the amygdala-cortical regions that underlie emotional face processing in adolescents with ASD relative to controls. There were three unique features of our fMRI task paradigm that facilitated the analysis to examine functional connectivity between these brain regions. First, we were able to control for potential group differences in attention to the emotional faces by instructing participants to make gender identification judgments. This ensured that participants attended to the emotional faces. Second, face stimuli were presented briefly for 250 ms, this limited the possibility that participants could make saccades away from the social stimuli. Thus, in order to perform the task accurately, participants in both groups had to attend to the faces during the full presentation duration. Third, the fMRI task was designed to elicit robust amygdala

activation in both groups. This enabled us to explore differences between groups in the correlational activity between the amygdala and cortical regions of interest.

We found less positive coupling between the amygdala and right middle temporal gyrus in the ASD group relative to the control group when participants viewed emotional faces (fearful, happy and sad) versus baseline. Similarly, we found less positive coupling between the amygdala and the inferior frontal gyrus in the ASD group relative to the control group when participants viewed sad faces versus baseline. Finally, consistent with our hypothesis, we found that less positive coupling between the amygdala and right middle temporal gyrus was correlated with a greater degree of social impairment within the ASD group. On the other hand, inconsistent with our hypothesis, we report greater positive coupling between the amygdala and inferior frontal gyrus was correlated with a greater degree of social impairment within the ASD group.

In sum, when the ASD and controls groups were both attending to emotional faces and engaging the amygdala equivalently, the ASD group showed less positive connectivity in cortical structures involved in face and emotion processing. Moreover, altered connectivity in the ASD group correlated with the core symptoms of social impairment.

Functional connectivity between the amygdala and right middle temporal gyrus

Our findings of less positive coupling between the amygdala and right middle temporal gyrus to emotional faces vs. baseline conditions in adolescents with ASD relative to controls are consistent with results from a previous study in our lab on adults with ASD (Monk, et al., *under review*). In this previous study, an attention cueing paradigm with emotional faces revealed less positive coupling between the amygdala and

the right middle temporal gyrus when adults with ASD relative to controls viewed sad pairs vs. neutral pairs. Consistent with our reports of less positive coupling between the amygdala and the right middle temporal gyrus in the ASD group relative to control groups, are reports of hypoactivation within a similar region of the middle temporal gyrus in individuals with ASD relative to controls in emotional face paradigms (Critchley, et al., 2000; Pelphrey, et al., 2007). It is important to note that other studies have also reported hypoactivation in the upper bank of the STS, namely the superior temporal gyrus (Wang, et al., 2007). Reports of dysfunction within these subregions of the STS might be due to differences in stimuli or baseline comparisons (Hein & Knight, 2008). Since the STS region is important for face processing and the amygdala is involved in communicating the emotional and social relevance of faces (Grelotti, Gauthier, & Schultz, 2002), a lack of synchrony between these two areas might provide further evidence as to why individuals with ASD might not extract the same amount of emotional information from the face as compared to typically developing individuals.

When the relationship between social severity and functional connectivity was examined, there was a significant negative correlation between the ADI-R current social score and strength of connectivity between the amygdala and the right middle temporal gyrus when participants viewed sad and happy faces relative to baseline. These findings indicate that adolescents with greater social impairments show reduced connectivity between the two regions. This finding is consistent with a prior study that reported a similar negative correlation between ADI-R social score and functional connectivity between the amygdala and the fusiform face area in adults with ASD (Kleinhans, et al., 2008). Although our findings are between the amygdala and the right middle temporal

lobe, it is important to note that the middle temporal gyrus extends posteriorly and may overlap with the fusiform face area reported in the Kleinhans et al., (2008) study. Consistent with our findings, another fMRI study reported that activity in the middle temporal gyrus was negatively correlated with the degree of social impairment when children and adolescents with ASD were given a task to interpret intentions through faces and voices (Wang, et al., 2007). There were several differences between our study and Wang et al., (2007). First, their analysis was not a functional connectivity analysis and therefore, their findings cannot be directly compared against ours. However, it is likely that lack of activation within a structure may be related to weaker functional connectivity. Second, they reported correlations with a different social measure, known as the SRS. In our study, covarying the SRS did not yield any findings that surpassed our threshold.

Functional connectivity between the amygdala and inferior frontal gyrus

In typically developing individuals, the inferior frontal gyrus (IFG) has consistently been activated when mirroring emotional facial expression (Carr, et al., 2003; Leslie, et al., 2004; Pfeifer, et al., 2008) and activation in the IFG correlates positively with degree of empathy (Hooker, et al., 2008; Saarela, et al., 2007). Therefore, there is evidence that this region is implicated in empathy. In addition, the amygdala has also been identified to work in tandem with the IFG to decipher emotional states of others (Pfeifer, et al., 2008). Indeed a PPI study involving emotional faces in typically developing individuals reported that there was a positive coupling between the amygdala and the IFG (Iidaka, et al., 2001). These findings might lend some support for the greater positive coupling between the amygdala and the IFG in our control group relative to the ASD group.

Our findings of weaker positive coupling between the amygdala and IFG in the ASD group relative to the control group in sad vs. baseline conditions are consistent with the Monk et al., (*under review*) adult study outlined above. In this previous study, a similar pattern of results was reported specifically in sad pairs vs. neutral pairs but not in other emotions (Monk, et al., *under review*). Research has shown that different emotions can elicit different physiological responses and evoke varying degrees of empathy (Wild, et al., 2001). Indeed, some authors have suggested that sad faces elicit "prosocial behavior" (Blair, et al., 1999). This might explain why we did not find a similar pattern in happy vs. baseline conditions.

Two recently published ASD studies are consistent with our present findings of less positive coupling in the ASD group relative to the control group within the IFG. Although they used different seed regions, they too reported less positive functional connectivity within the IFG in the ASD group relative to controls in response to tasks with various social stimuli (Kleinhans, et al., 2008; Koshino, et al., 2008).

Since ASD is associated with deficits in social cognition and feelings of empathy (Iacoboni & Dapretto, 2006), and a notable study by Dapretto et al. (2006) found that reduced activation in the IFG was correlated with poorer social functioning, our findings of less positive connectivity between the amygdala and IFG might suggest that the pathways between these two structures might be compromised in ASD, as these structures might not work in concert with one another. In order to determine if adolescents with greater impairment exhibited less synchrony within empathy related regions in our ASD group, we performed a follow-up analysis to elucidate whether the degree of social impairment correlated with functional connectivity.

Contrary to our predictions, the ADI-R ever social score showed a significant positive correlation with the strength of connectivity between the amygdala and the IFG when participants viewed sad and happy faces relative to baseline, indicating that adolescents with greater social impairments showed greater positive coupling between the two regions. This unexpected positive correlation within the inferior frontal gyrus was also noted in a prior study (Kleinhans, et al., 2008). However, there were several differences between this study and ours. First, the seed region was in the fusiform face area and not in the amygdala. Second, the social measure that Kleinhans et al., (2008) used was derived from the ADOS and not the ADI-R "ever" score. Because the ADI-R "ever" score is computed by considering social impairment seen throughout the individual's life, it therefore might not reflect the individual's current social abilities. In our analysis, covarying the other measures of social ability such as the ADOS, ADI-R current score and SRS did not yield any findings that surpassed our threshold.

Emotional Recognition behavioral task

The emotional recognition task administered after the MRI scan enabled us to evaluate whether there were group differences in performance when participants were asked to identify the facial emotions used in the fMRI task. We noted that adolescents with ASD were just as accurate and as fast as controls in recognizing emotional expressions (refer to Table 2.3). There were several possible reasons why we did not observe group differences in performance and mean reaction time. First, our N was small and this could have obscured potential differences in performance between groups. Second, our task might not have been sensitive enough to capture subtle differences between groups in identifying facial expressions. For example, tasks that found

differences in emotional recognition often used facial stimuli, which showed a gradient or varying degree of facial expressions that enabled them to pick up subtle differences (Humphreys, et al., 2007). In contrast, the facial stimuli that were utilized in our task were unambiguous and easy to identify, as they consisted of basic emotions. Thirdly, some authors have suggested that by the time individuals with ASD reach adolescence, they are able to tell apart different emotions (B. Wright, et al., 2008). In line with this argument, and consistent with our behavioral findings, some authors whose ASD group comprised of individuals who had similar demographics (IQ > 85) and ages did not find group differences in emotional recognition abilities (B. Wright, et al., 2008). Therefore, it seems likely that the less positive connectivity observed in our ASD group relative to the control group were not due to differences in emotional face recognition abilities. Instead, these findings could reflect differences of brain connectivity between the ASD and control groups in networks that underlie emotional face processing.

Limitations

There were several limitations to the study. First, our sample size was relatively small. However, since small sample sizes reduce power, the findings in this present chapter appear to reflect relatively large effect sizes. Second, our ASD group consisted solely of high functioning adolescents. Therefore, the findings here reflect neural patterns in a subpopulation of ASD and might not be generalizable to individuals at lower ends of the autism spectrum. Third, gender identification judgments made it difficult to tease apart group differences that stemmed from these cognitive judgments versus those which stemmed from viewing the emotional faces. Some authors have suggested that this poses potential problems (Schulte-Ruther, et al., 2007). However, since there were no marked

differences between the two groups in neutral face conditions on either response time or accuracy, this suggests that group differences seen in the other face conditions were likely due to the emotional content of the faces. Finally, as other authors have suggested, PPI is a limited technique, as it cannot determine the direction of modulation (Foland, et al., 2008) but can only provide information about whether the time courses between two regions vary positively or negatively within a specific experimental condition when comparing both groups. However, despite this limitation, the synchrony between brain regions is a valuable tool in providing information of how neural networks functions as a whole.

Implications and future directions

Additional work needs to be carried out to elucidate the role of the amygdala in face processing as neuroimaging results have been mixed. First, some have suggested that differences in amygdala activation are due to the inability to control for attention between groups (Dalton, et al., 2005; Monk, et al., *under review*) and that when gaze fixation, an index for attention was considered, individuals with ASD elicited robust amygdala activation (Dalton, et al., 2005). In the present study, the brief presentation duration may have played a role in the absence of group differences in amygdala activation. Future studies could vary presentation duration to examine the effects of amygdala function in ASD and control samples. Second, in addition to task differences, discrepancies in amygdala activation could be due to varying levels of development and functioning across samples. Few studies have examined amygdala function in adolescents with ASD. Indeed, structural studies in typically developing and ASD individuals show tremendous changes in group differences during development (Nacewicz, et al., 2006; Schumann, et

al., 2004; Sparks, et al., 2002). Thus, our present findings for an absence of group differences in amygdala activation may be a function of the developmental period under investigation. Future studies examining changes in amygdala function with age in normative development as well as in ASD are needed to better characterize the role of the amygdala in emotional processing.

Conclusions

The findings of this study provide evidence that adolescents with ASD show disturbances in functional connectivity within the networks involved in emotional face processing. Notably, adolescents with ASD relative to controls showed less positive functional connectivity between the amygdala and the right middle temporal and the inferior frontal gyrus. In addition, the degree of social impairment varied with the strength of the connectivity between the amygdala and these cortical areas. It will be useful to explore white matter connections between the amygdala and regions of the right temporal and inferior frontal cortices, using techniques such as diffusion tensor imaging (DTI). In addition, further investigations should focus on documenting disturbances in functional connectivity at different stages of development. This will allow further elucidation of disturbances within neural networks that underlie emotional face processing in ASD.

TABLES

Table 2.1: Subject characteristics

	ASD	**Control**	***t* (df)**	***p* value**
Age, mean (SD)	14 (1.40)	15 (1.42)	1.21 (25)	0.24
Age range	13 - 17	13 - 18		
Male to female ratio	12:01	13:01		
Verbal cognitive functioning, mean (SD)	112 (18.65)	116 (11.46)	0.72 (25)	0.48
Non-verbal cognitive functioning, mean (SD)	115 (11.86)	108 (10.20)	1.60 (25)	0.12
Handedness left to right ratio	2:11	1:13		

Table 2.2: Functional MRI face task performance and reaction time measures across emotion. No significant differences in accuracy or reaction time were found between the ASD and the control groups.

Emotion		ASD Mean (SD)	Control Mean (SD)	*t* (df)	*p* value
Fearful	Accuracy (%)	94.87 (4.22)	97.14 (3.42)	1.54 (25)	0.14
	RT(ms)	738.00 (139.32)	688.45 (116.77)	1.00 (25)	0.32
Happy	Accuracy (%)	94.87 (5.55)	97.14 (3.66)	1.26 (25)	0.22
	RT(ms)	732.08 (135.95)	689.75 (107.24)	0.90 (25)	0.38
Sad	Accuracy (%)	95.13 (4.22)	96.43 (4.62)	0.76 (25)	0.45
	RT(ms)	747.91 (152.30)	695.20 (115.10)	1.11 (25)	0.32
Neutral	Accuracy (%)	96.15 (2.67)	96.90 (3.80)	0.59 (25)	0.56
	RT(ms)	728.95 (127.63)	698.53 (108.48)	0.67 (25)	0.51

Table 2.3: Emotional recognition task performance and reaction time measures across emotion. No significant differences in accuracy or reaction time were found between the ASD and the control groups.

Emotion		ASD Mean (SD)	Control Mean (SD)	*t* (df)	*p* value
Fearful	Accuracy (%)	81.27 (17.13)	86.62 (8.66)	1.04 (25)	0.31
	RT(ms)	1288.38 (323.07)	1326.43 (211.27)	0.37 (25)	0.72
Happy	Accuracy (%)	96.41 (4.99)	94.24 (8.41)	0.81 (25)	0.43
	RT(ms)	1053.69 (241.63)	1057.71 (198.52)	0.05 (25)	0.96
Sad	Accuracy (%)	91.72 (9.47)	92.12 (4.27)	0.14 (25)	0.89
	RT(ms)	1169.46 (256.54)	1042.64 (156.51)	1.56 (25)	0.13
Neutral	Accuracy (%)	87.69 (6.99)	89.05 (8.62)	0.45 (25)	0.66
	RT(ms)	1288.46 (343.68)	1099.00 (195.11)	1.78 (25)	0.09

Table 2.4: Left and right amygdala in ASDs and controls. Displayed are the *T*-values and xyz coordinates for the local peaks within the amygdala for each contrast. *$p < 0.05$ (SVC corrected), *N.S* stands for non significant.

	ASD		CONTROLS		ALL	
Condition	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
Fearful vs. Baseline	*T=3.26; -20 -8 -14	*N.S*	*T=6.49; -20 -8 -12 *T=5.47; -26 -2 -14	*T=5.82; 18 -8 -14	*T=4.80; -20 -8 -14 *T=3.16; -30 -2 -18	*T=3.88; 18 -8 -14 *T=2.96; 30 -2 -20
Happy vs. Baseline	*T=3.23; -22 -8 -10	*N.S*	*T=4.49; -22 -8 -10 *T-4.27; -22 -4 -14	*N.S*	*T=4.14; -22 -8 -10	*N.S*
Sad vs. Baseline	*T=3.73; -20 -2 -14 *T=3.23; -22 -8 -12	*N.S*	*T=6.28; -22 -8 -12 *T=6.17; -20 -2 -14 *T=3.92; -30 -2 -22 *T=2.88; -26 -4 -26	*T=3.23; 26 2 -20 *T=2.87; 28 -2 -14 *T=2.82; 24 -8 -10 *T=2.74; 22 -2 -14	*T=5.13; -20 -2 -14 *T=4.77; -22 -8 -12 *T=3.34; -30 -4 -22 *T=2.78; -26 -4 -26	*T=3.06; 24 -8 -10 *T=2.79; 26 2 -20
Neutral vs. Baseline	*T=3.66; -20 -2 -14 *T=3.33; -20 -8 -10	*N.S*	*T=8.03; -24 -2 -14 *T=5.37; -22 -8 -10	*T=3.78; 22 -2 -14 *T=3.66; 24 -4 -20 *T=3.27; 22 -8 -10	*T=5.37; -22 -2 -14 *T=4.51; -22 -8 -10	*T=2.76; 24 -4 -20

FIGURES

Figure 2.1: Temporal display of the fMRI task paradigm. The ITI was jittered and ranged from 0ms to 6000ms at intervals of 2000ms. The ITIs were distributed equally but appeared in random order.

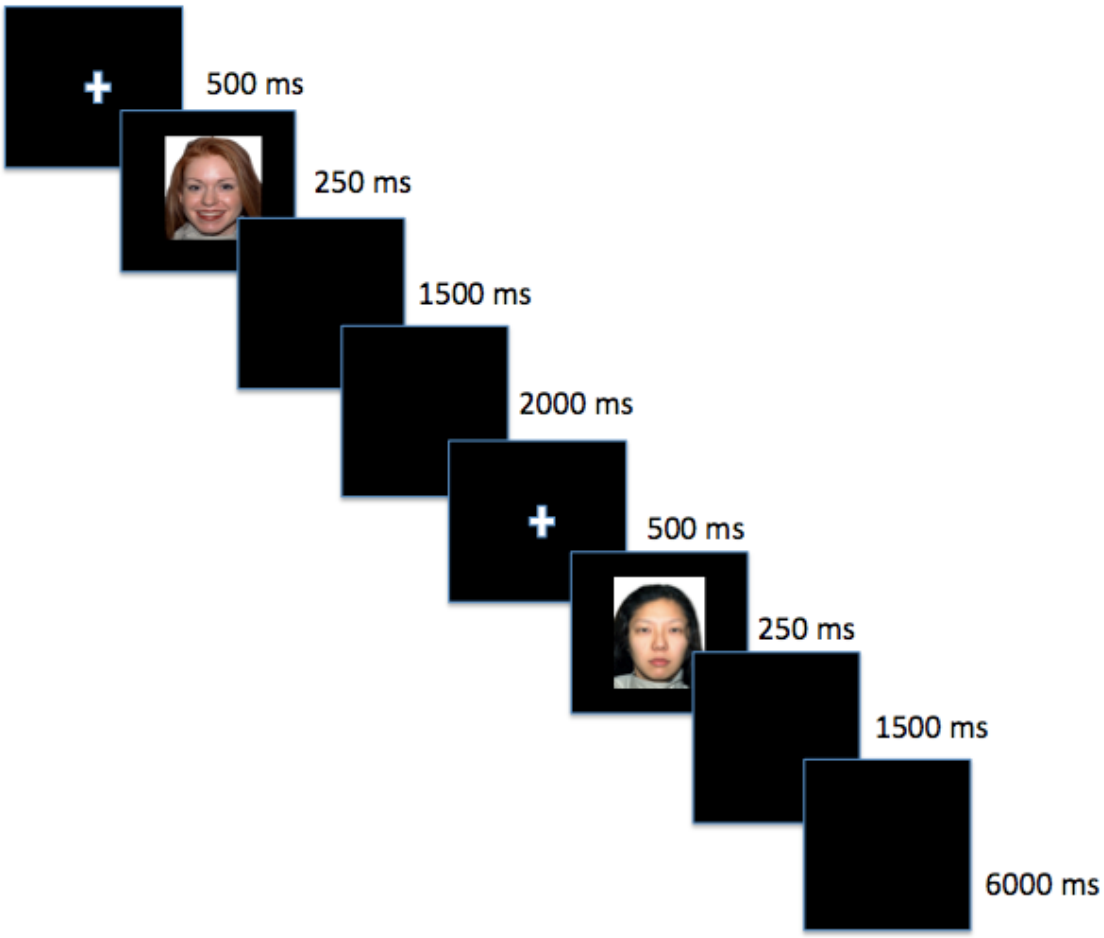
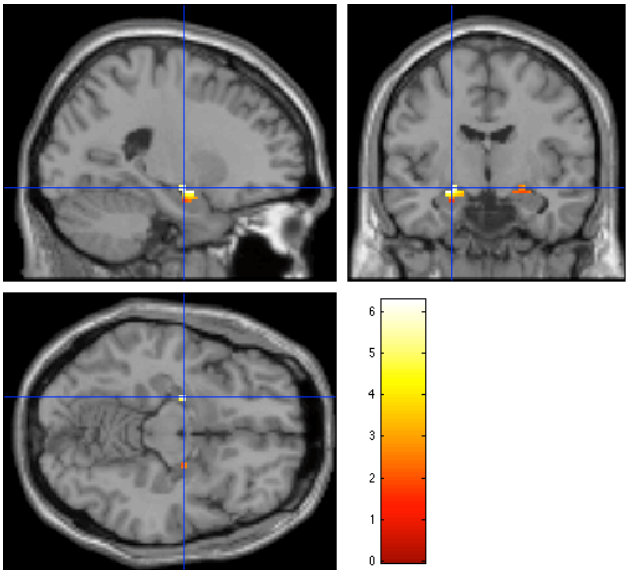


Figure 2.2: Bilateral amygdala activation in the control and the ASD group in sad vs. baseline. For illustration purposes, a threshold of $p < 0.05$ (uncorrected) and a bilateral amygdala mask was used. Figure 2A: Cross hair shows peak activation in the left amygdala in controls, $t(13) = 6.28$, $p < 0.001$ (SVC corrected), xyz = -22 -8 -12. Activation in the right amygdala was also noted, $t(13) = 3.23$, $p = 0.023$ (SVC corrected), xyz = 26 2 -20. Figure 2B: Cross hair shows peak activation in the left amygdala in the ASD group, $t(12) = 3.73$, $p = 0.008$ (SVC corrected), xyz = -20 -2 -14. A trend for activation in the right amygdala was also noted but was not significant at the SVC correction level.

A.



B.

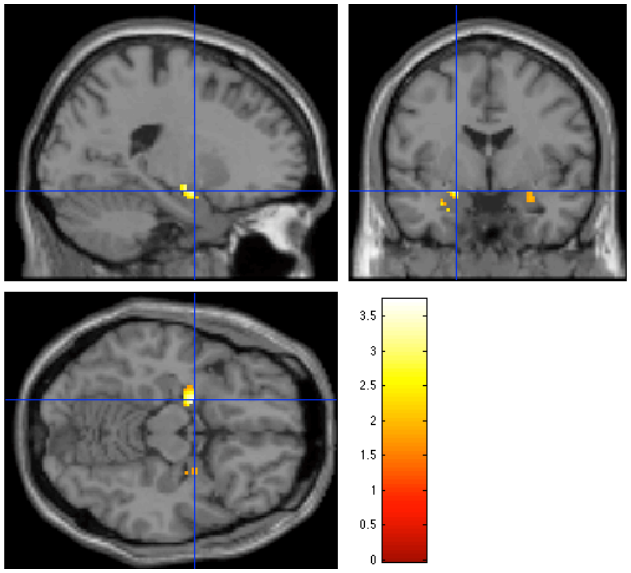


Figure 2.3: Controls relative to ASDs showed greater positive coupling between the left amygdala and the right middle temporal gyrus to fearful vs. baseline, $t(25) = 5.02$, $p = 0.004$ (SVC corrected), xyz = 46 -48 -8. For illustration purposes we displayed cluster size, $k > 50$ voxels with a threshold at $p < 0.05$.

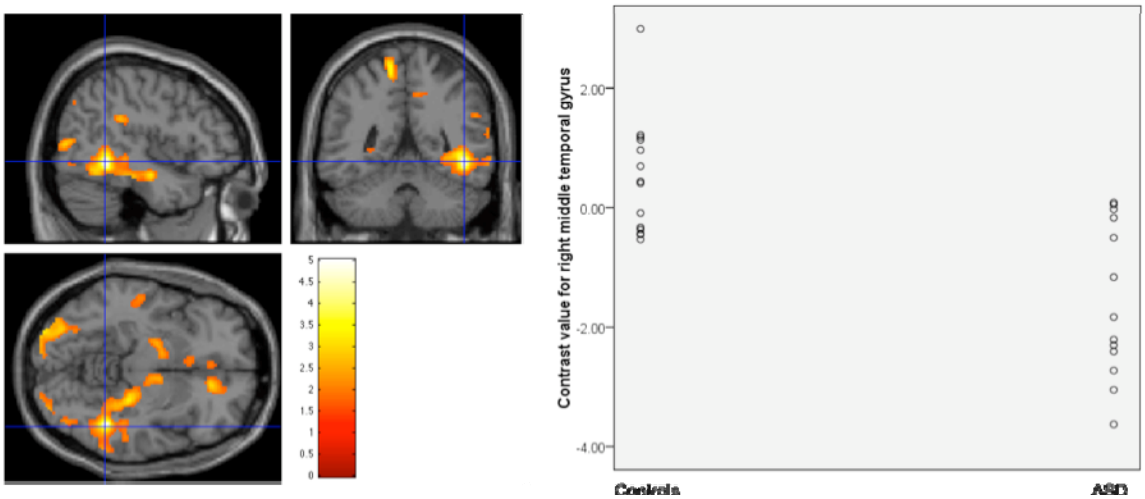


Figure 2.4: Controls relative to ASDs showed greater positive coupling between the right amygdala and the right middle temporal gyrus to sad vs. baseline, $t(25) = 3.72$, $p = 0.042$ (SVC corrected), xyz = 44 -72 6. For illustration purposes we displayed cluster size, $k > 50$ voxels with a threshold at $p < 0.05$.

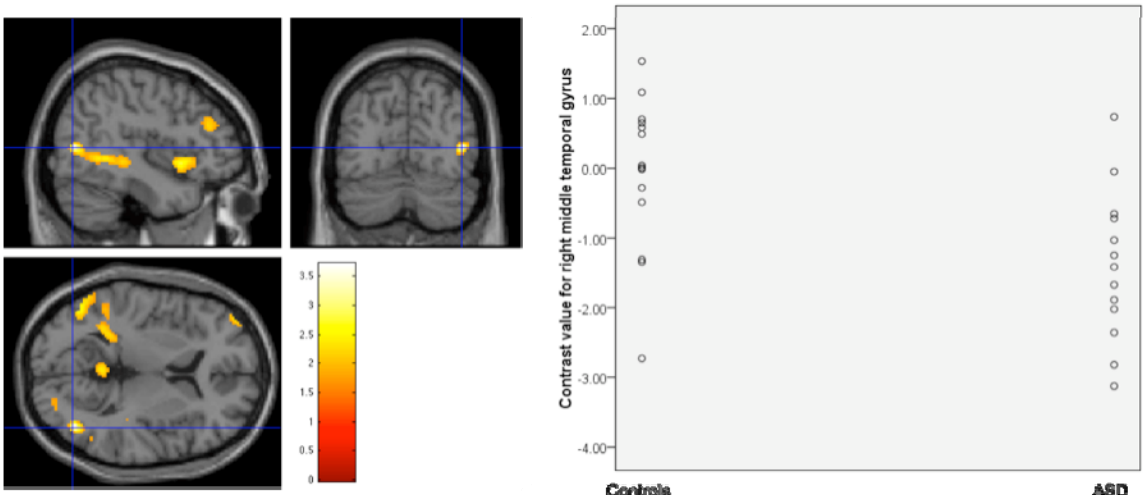


Figure 2.5: Controls relative to ASDs showed a trend for greater positive coupling between the left amygdala and the right middle temporal gyrus in happy vs. baseline, $t(25) = 3.46$, $p = 0.001$ (uncorrected), xyz = 48 -30 -16. For illustration purposes we displayed cluster size, $k > 50$ voxels with a threshold at $p < 0.05$.

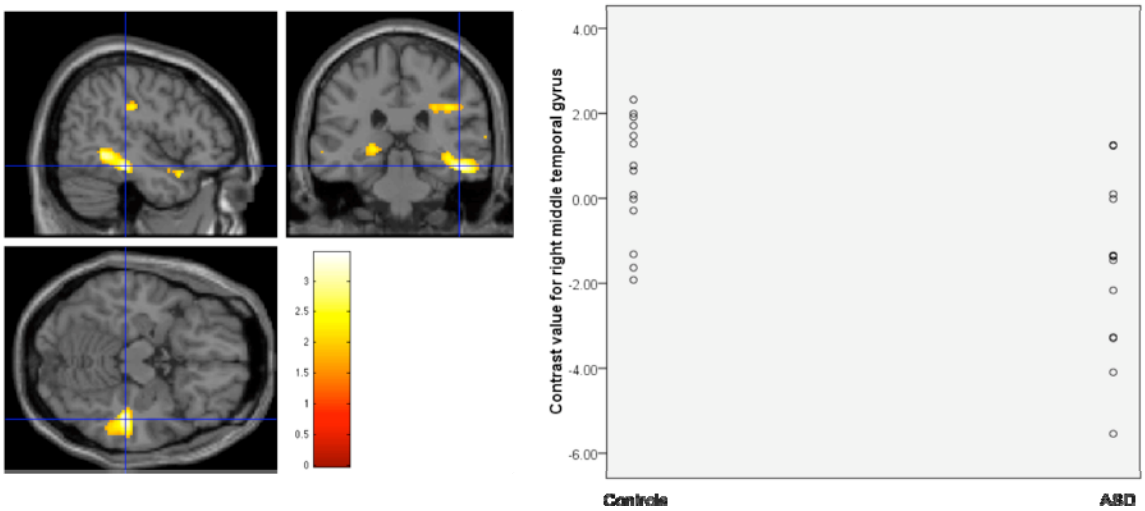


Figure 2.6: Controls relative to ASDs showed greater positive coupling between the left amygdala and the right inferior frontal gyrus to sad vs. baseline, $t(25) = 3.88$, $p = 0.042$ (SVC corrected), xyz = 40 32 -16. For illustration purposes we displayed cluster size $k >$ 200 voxels with a threshold at $p < 0.01$.

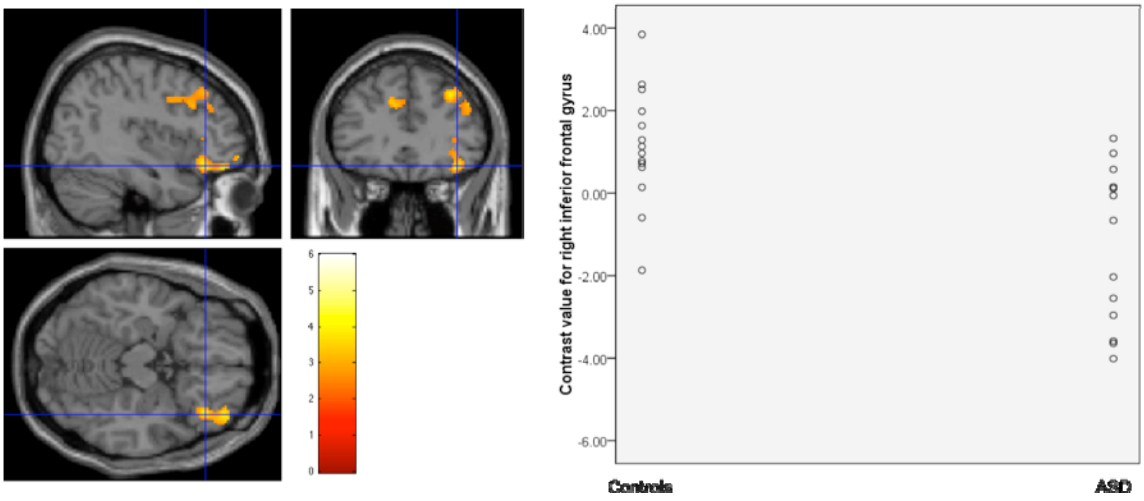


Figure 2.7: Controls relative to ASDs showed a trend for greater positive coupling between the right amygdala and the left inferior frontal gyrus to sad vs. baseline, $t(25) = 3.50$, $p = 0.001$ (uncorrected), xyz = -40 34 -6. For illustration purposes we displayed cluster size $k > 200$ voxels with a threshold at $p < 0.01$.

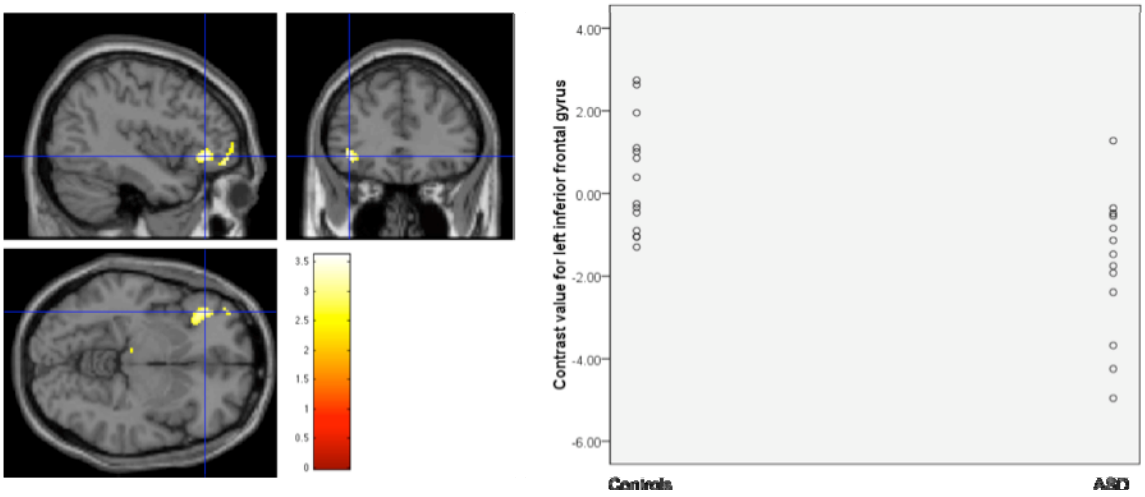


Figure 2.8: Within the ASD group, social functioning based on the ADI measure of total reciprocal social interaction (current), showed a negative correlation with functional connectivity between the left amygdala and the right middle temporal gyrus in the happy vs. baseline condition, $t(11) = 3.77$, $p = 0.002$, xyz = 50 -56 -8. For figure 2.8A, the threshold was set at $p < 0.05$. To illustrate this association, contrast values were extracted from a 4mm sphere around the peak activation and plotted with the ADI measure of social function, Pearsons $r = -0.70$, $p = 0.007$ (figure 2.8B).

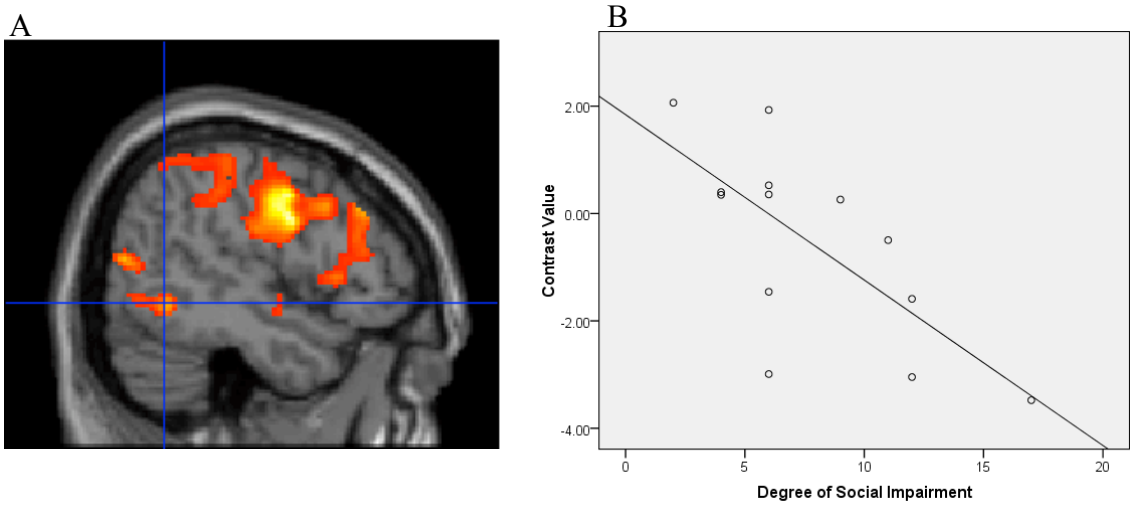
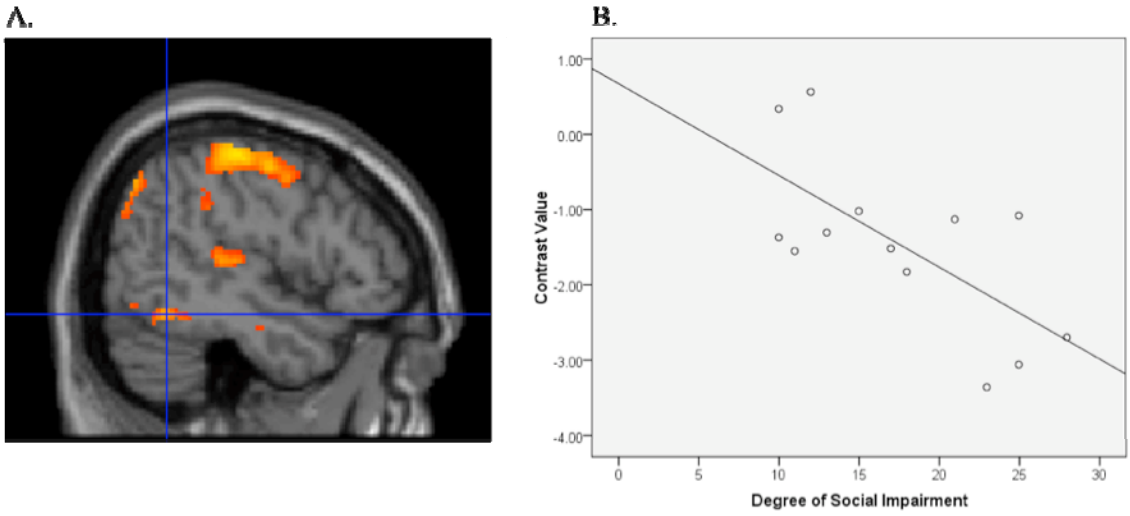


Figure 2.9: Within the ASD group, social functioning based on the ADI measure of total reciprocal social interaction (ever), showed a negative correlation with functional connectivity between the left amygdala and the right middle temporal gyrus in the fearful vs. baseline condition, $t(11) = 3.00$, $p = 0.006$, xyz = 50 -52 -16. For figure 2.9A, the threshold was set at $p < 0.05$. To illustrate this association, contrast values were extracted from a 4mm sphere around the peak activation and plotted with the ADI measure of social function, Pearsons $r = -0.67$, $p = 0.012$ (figure 2.9B).



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CHAPTER III

DISTURBANCES OF INTRINSIC FUNCTIONAL CONNECTIVITY IN THE DEFAULT NETWORK IN ADOLESCENTS WITH AUTISM SPECTRUM DISORDERS

ABSTRACT

Autism spectrum disorders (ASD) are associated with disturbances of neural connectivity. Connectivity is typically examined within the context of a cognitive task. However, connectivity also exists in the absence of a task. This intrinsic connectivity, known as resting-state connectivity, is particularly active in a set of structures called the default network, which includes the posterior cingulate cortex (PCC), retro-splenial cortex, lateral parietal cortex/angular gyrus, medial prefrontal cortex, superior frontal gyrus, temporal lobe, and parahippocampal gyrus. Exploring functional connectivity within the default network is of interest in ASD since this networks has been suggested to be involved in low-level monitoring of the environment, contemplating future scenarios, and in the homeostasis of excitatory and inhibitory neuronal inputs. Following previous seminal studies on the default network, when a seed in the PCC was used to examine pairwise couplings in the default network, a previous resting connectivity study in our lab showed patterns of weaker connectivity/coupling between the PCC and the right superior frontal gyrus and tighter connectivity/coupling between the PCC and the right superior temporal gyrus as well as between the PCC and the right parahippocampal gyrus in adults with ASD relative to controls. Therefore, in the present study, we first hypothesized that

adolescents with ASD would show weaker coupling between the PCC and right superior frontal gyrus relative to controls. Second, we hypothesized that adolescents with ASD would show tighter coupling between the PCC and the right superior temporal gyrus and between the PCC and right parahippocampal gyrus relative to controls. Third, we included an exploratory analysis in which we hypothesized that adolescents with ASD relative to controls would show evidence for group differences in the default network in addition to what was previously found in the adult sample. Finally, we hypothesized that the strength of connectivity within the default network would relate to clinical measures such as symptom severity and adaptive functioning within the ASD group. **Methods**: 12 adolescents with ASD and 12 controls between the ages of 13-17 took part in a functional MRI study. Participants were instructed to "let your mind wander freely" while looking at a fixation cross displayed in the middle of the screen for 10 minutes during fMRI acquisition. A seed region was placed in the PCC and functional connectivity was examined by obtaining the correlational activity between the PCC and other areas of the default network. **Results:** Both the ASD and control groups activated the default network of the brain at $p<0.05$ (whole brain corrected). Analyses of group differences revealed that adolescents with ASD relative to controls showed weaker coupling between the PCC and the right superior frontal gyrus (corrected for multiple comparisons). In addition, adolescents with ASD relative to the controls showed tighter coupling between the PCC and the right superior temporal gyrus. As predicted our findings in the adolescent sample differed from what was reported in the adult sample. Specifically, weaker coupling in adolescents with ASD relative to controls was not confined to the right superior frontal gyrus, but instead was evident in a majority of areas in the default network. Moreover,

poorer adaptive behavioral skills correlated with weaker connectivity between the PCC and the left angular gyrus. **Conclusions:** These findings indicate that adolescents with ASD show evidence for altered intrinsic connectivity within the default network. In addition, we report that a majority of the pairwise couplings between the PCC and other areas of the default network show weaker connectivity in adolescents with ASD than what was previously reported in adults with ASD. Finally, we provide evidence that weaker connectivity within the default network may underlie impairments seen in ASD.

INTRODUCTION

Individuals with autism spectrum disorders (ASD) suffer from widespread deficits in the social domain and these deficits are often accompanied by language delays and difficulties in social communication. In addition, many individuals with ASD often display restricted and repetitive behaviors and or interests (RRB). These difficulties can have a profound impact on an individuals overall level of adaptive functioning, especially in their daily interactions with others.

Converging lines of evidence support the claim that ASD is a disorder of connectivity (Belmonte, et al., 2004; Just, Cherkassky, Keller, Kana, & Minshew, 2007). First, at the neural level, individuals with ASD show marked disturbances in cortical organization as evidenced by narrower and more densely packed columns of neuronal cells (Casanova, et al., 2006). Second, neuroanatomical studies have reported region specific white matter growth in children with ASD (Herbert, et al., 2004) and this has been interpreted as evidence to support the idea that ASD is characterized by increases in short to medium range intrahemispheric connections and fewer longer range interhemispheric connections (Herbert, et al., 2003; Herbert, et al., 2004). In addition, diffusion tensor imaging (DTI) techniques have found that white mater integrity is compromised in ASD (Alexander, et al., 2007; Barnea-Goraly, et al., 2004). Third, the field of neuroimaging has revealed abnormalities in functional connectivity within regions of the brain in ASD. There have been reports that individuals with ASD relative to controls show patterns of tighter connectivity (Mizuno, Villalobos, Davies, Dahl, & Muller, 2006; Turner, Frost, Linsenbardt, McIlroy, & Muller, 2006) and reports that show patterns of weaker connectivity (Just, et al., 2007; Kana, Keller, Cherkassky, Minshew, &

Just, 2006; Kleinhans, et al., 2008; Koshino, et al., 2008; Villalobos, Mizuno, Dahl, Kemmotsu, & Muller, 2005; Welchew, et al., 2005; Wicker, et al., 2008). All these lines of evidence suggest that there is profound disruption in brain connectivity in ASD.

The functional connectivity studies reported above have traditionally been carried out in the presence of a cognitive task. However, recent studies have established that there is a set of brain regions, known as the default network, that are active even in the absence of a task (Buckner & Vincent, 2007; Raichle & Snyder, 2007). The brain regions that form the default network are the posterior cingulate cortex (PCC), retrosplenial, lateral parietal/angular gyrus, medial prefrontal cortex, superior frontal gyrus, regions of the temporal lobe, and finally the parahippocampal gyrus (Fox, et al., 2005; Greicius, Krasnow, Reiss, & Menon, 2003; Shulman, et al., 1997). Intrinsic activity of these brain regions has been identified to be active when individuals are awake (Fox, et al., 2005; Greicius, et al., 2003), asleep (Fransson, et al., 2007; Fukunaga, et al., 2006; Redcay, Kennedy, & Courchesne, 2007) or under anesthesia (Kiviniemi, et al., 2000). Because of the tremendous amount of energy that this intrinsic activation consumes in comparison to what is consumed when the brain is engaged in a task (Raichle & Mintun, 2006), authors posit that intrinsic activation may extend beyond thought processes and encompass the role of maintaining homeostasis between excitatory and inhibitory neuronal responses (Biswal, Yetkin, Haughton, & Hyde, 1995; Laughlin & Sejnowski, 2003) as well as contemplating scenarios and events or lower-level observations of the individuals external surroundings (Buckner, Andrews-Hanna, & Schacter, 2008; Raichle & Snyder, 2007). Other authors have proposed that the default network might be active during self-referencing and introspection (Iacoboni, 2006) and that dysfunction within the default

network, which subserves aspects of social cognition and empathy might account for impairments seen in ASD (Kennedy & Courchesne, 2008).

A large majority of studies have focused on examining the default network in typically developing adult populations (Fox, et al., 2005; Greicius, et al., 2003; Greicius, Supekar, Menon, & Dougherty, 2009). It was not until recently, that studies have begun to uncover abnormalities in functional connectivity within the default network in individuals with ASD (Cherkassky, Kana, Keller, & Just, 2006; Kennedy & Courchesne, 2008; Monk, et al., *under review*). With the exception of the Cherkassky et al., (2006) study, which reported widespread decreases in connectivity, both the Kennedy and Courchesne, (2008) and the Monk et al., (*under review)* study showed alterations in specific areas of the default network. However, all of these studies have been carried out in adults with ASD and no known study has examined the default network in adolescents with ASD.

Adolescence is characterized as a period in which dynamic changes in the brain occur. There have been a substantial number of studies that have confirmed that there are dramatic anatomical changes that occur within the brain during this period of life (Giedd, 2008; Sowell, et al., 2003). There is also reason to believe that these structural changes can often be accompanied by functional changes within specific brain regions (Booth, et al., 2001; Koch, Norris, & Hund-Georgiadis, 2002). Indeed, a study has shown that functional connectivity within the default network is more loosely connected at younger ages in typically developing populations (Fair, et al., 2008). Similar to their typically developing counterparts, adolescents with ASD are likely to show age related changes within the default network. Examining the default network in adolescents with ASD would enable better characterization of abnormal connectivity seen in ASD throughout

development and will allow us to explore regions of the default network that relate to impairments in the social, communication, restricted and repetitive behavioral (RRB) domains, as well as the individuals level of adaptive behavior.

Goals of the study

The goal of this study was to examine the default network in adolescents with ASD and to examine how adaptive behavior and severity of symptoms relate to brain function. To evaluate functional connectivity in the default network, we employed the same paradigm as Monk et al., (*under review*) and monitored the default network for 10 minutes in adolescents with ASD in the absence of a task. Specifically, a seed in the posterior cingulate cortex (PCC) was used to examine pairwise couplings between the PCC and each area of the default network. Past studies have used this seed successfully in adults (Fox, et al., 2005; Monk, et al., *under review*; Shulman, et al., 1997) and children (Thomason, et al., 2008) to reveal connectivity in the default network. This method enabled us to examine where adolescents with ASD exhibited deviations in connectivity. First, following prior reports of weaker connectivity/coupling between the PCC and the right superior frontal gyrus and tighter coupling between the PCC and the right superior temporal as well as the PCC and the right parahippocampal gyrus in adults with ASD relative to controls (Monk, et al., *under review*), we hypothesized that adolescents with ASD would also show weaker coupling between the PCC and the right superior frontal gyrus relative to controls. Second, we hypothesized that adolescents with ASD would also show tighter coupling between the PCC and the right superior temporal gyrus as well as the PCC and the right parahippocampal gyrus. Third, since there have been reports that the default network is more loosely connected at younger ages in typically developing

populations, we hypothesized that adolescents with ASD relative to controls would show evidence for group differences in the default network in addition to what was previously found in an adult sample. Finally, we hypothesized that adaptive behavior and severity of symptoms within the ASD group would correlate with connectivity within the default network.

METHODS

Participants

Sixteen high functioning adolescents with ASD (IQ>85) and 14 healthy controls participated in the study. Due to excessive movement, 2 participants were removed (1 ASD and 1 control). In addition, 3 adolescents with ASD could not complete the resting connectivity scan due to nervousness and anxiety related issues. Lastly, 1 control was excluded due to technical complications that arose during data preprocessing. The final set consisted of 12 adolescents with ASD and 12 controls. The ASD group consisted of 11 males and 1 female between 13 to 17 years of age and the control group consisted of 11 males and 1 female between the 13 to 18 years of age. Of the 12 adolescents with ASD, 4 were diagnosed with autism, 1 was diagnosed with Asperger syndrome and 7 were diagnosed with pervasive developmental disorder not otherwise specified (PDD-NOS). Adolescents with ASD were recruited through the University of Michigan Autism and Communication Disorders Center (UMACC) and received their diagnosis 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 confirmed by clinical consensus. Controls were recruited through posted flyers and advertisements and were excluded if diagnosed with any mental or neurological

condition. Verbal and non-verbal cognitive functioning was obtained by administering the Peabody Picture Vocabulary Test (PPVT) (Dunn & Dunn, 1997) and the Ravens Progressive Matrices (Raven, 1960) respectively. In the ASD group, 7 of the 12 ASD participants were taking psychotropic medication (2 were on selective serotonin reuptake inhibitors, 5 were on stimulants, 3 were on neuroleptics and 1 was on atomoxetine). Post-hoc analyses were carried out to determine if the medications contributed to group differences (refer to Results). There were no significant group differences in age, verbal and nonverbal cognitive functioning, gender, and handedness (refer to Table 3.1).

Procedures

The University of Michigan Institutional Review Board approved all procedures. All participants underwent an initial phone screening to ensure that none of the participants had surgeries in which metal was placed in the body. In addition, we did not recruit adolescents who wore braces as the metal can interfere with fMRI acquisition. With regards to the ASD group, we screened out adolescents who had co-occurring psychiatric disorders and history of seizures.

Participants were scheduled for an initial visit prior to the fMRI scan visit. During the first visit, parents signed consent forms and completed parent questionnaires and the adolescents signed assent forms and completed self-report questionnaires to gain a better understanding of overall functioning. In addition, we obtained levels of social functioning using the Social Responsiveness Scale (SRS) (Constantino, et al., 2003) and Social Communication Questionnaire (SCQ) (Rutter, et al., 2003) within both groups so as to ensure that the control group did not display social functioning scores within the ASD range. Finally, participants were familiarized to the fMRI procedures by having them lie

in a mock MRI for several minutes. During the second visit, participants underwent an fMRI scan at the University of Michigan fMRI lab. During this visit, participants were screened for the presence of metal in their bodies prior to entering the fMRI scanner. The fMRI scan lasted for approximately 45 minutes.

fMRI Data Acquisition

Participants lay supine in the fMRI scanner and wore goggles with built-in mirrors (VisuaStim XGA, Resonance Technologies) in order to view the projected stimuli inside the scanner. A black fixation cross on a white background was displayed in the center of the screen for 10 minutes. Participants were instructed to keep their eyes open and fixed on the cross. In addition, participants were told explicitly to "let their minds wander freely" and to not dwell on anything in particular. A pulse oximeter was attached to the participant's finger in order to obtain their cardiac response. In addition, a pressure belt was worn around the participant's abdomen in order to obtain their respiratory response. Both the cardiac and respiratory signals were synchronized to the fMRI data.

Imaging was performed on a long bore 3T GE signa scanner operating on a 12.0 platform at the University of Michigan's fMRI lab. A GE quad head coil was used. For the functional data, a total of 300 T2* weighed BOLD images were acquired using a reverse spiral sequence (Glover & Law, 2001). Whole brain coverage was obtained with 40 contiguous 3mm axial slices (TR=2000 ms, TE=30 ms, flip angle=90°, FOV=22 cm, 64x64 matrix). Each slice was acquired parallel to the AC-PC line. Structural data included two T1 weighted images. The first was a 3D T1 axially acquired anatomical localizer 3D (TR=8.9, TE=1.8, flip angle=15°, FOV=26 cm, slice thickness=1.4 mm, 124 slices; matrix=256 x160). The second was a sagitally acquired high-resolution spoiled

gradient- recalled acquisition in stead state (SPGR) image (flip angle=15°, FOV=26cm, 1.4mm slice thickness, 110 slices).

Preprocessing of fMRI data

Initial preprocessing steps were carried out at the University of Michigan's fMRI lab. These included removing k-space outliers in raw data that were two standard deviations away from the mean and substituting them with the average value from neighboring voxels. Next, a field map correction was performed on the reconstructed images to remove the distortions that resulted from magnetic field inhomogenity. The variance due to physiological (cardiac and respiratory signals) responses was removed using a regression analysis (Glover, Li, & Ress, 2000). The data were then slice-time corrected using local sinc interpolation(Oppenheim, Schafer, & Buck, 1999) and realigned using McFlirt in FSL (Jenkinson, Bannister, Brady, & Smith, 2002).

After initial preprocessing steps were carried out, the functional images were first examined to exclude cases with head motion greater than 3mm in any of the six motion parameters. Additional preprocessing and image analysis were performed in SPM2 (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk). First we coregistered the high-resolution T1 images to the functional images. Second, T1 images were normalized to the scalped T1 template in SPM2 and the functional volumes were normalized using a similar transformation matrix. Third, images were smoothed using an isotropic 8mm full- width-half maximum (FWHM) Gaussian kernel.

A regression analysis was performed prior to generating the functional connectivity maps in order to reduce the noise related to movement. This was done by

entering the 6 motion parameters as nuisance covariates for each individual subject. In order to create functional connectivity maps, the data was passed through 3 in-house batch scripts implemented in MATLAB 7.0 (The Mathworks Inc. Natick, MA). The first script was used to low-pass filter the data at 0.08 Hz to remove higher frequency sources of noise (Biswal, et al., 1995). The second script, placed a seed region in the posterior cingulate cortex that was centered at -5 -53 41 (MNI). This seed region was employed following previous publications (Fox, et al., 2005; Shulman, et al., 1997). The seed region that was used in these studies were reported in Talairach-Tournoux space and corresponded to -5 -49 40 (TAL). A 4-voxel square was centered around the seed. The third script utilized the average seed region timecourse to correlate it with other pixels to generate functional connectivity maps for each individual subject.

We performed a second-level random effects analysis in SPM2 in order to examine the differences between the ASD and the control group. Regions of interest (ROI) were determined using WFU Pickatlas toolbox (http://www.fmri.wfubmc.edu/) (Maldjian, Laurienti, Burdette, & Kraft, 2002) labeled regions.

We used a threshold of $p<0.05$ family-wise error correction for examining the default network within the ASD groups and control groups separately. Following seminal default network studies (Fair, et al., 2008; Fox, et al., 2005), there were a total of 11 regions in which we examined functional connectivity with the PCC seed region, resulting in 11 pairwise couplings that were examined. The 11 regions were the (bilateral retro-splenial/BA30; left lateral parietal/ angular gyrus; right lateral parietal/angular gyrus; left medial prefrontal/BA32 and BA10 combined; right medial prefrontal/BA32 and BA10 combined; left superior frontal gyrus; right superior frontal gyrus; left

temporal lobe; right temporal lobe; left parahippocampal gyrus and the right parahippocampal gyrus. In order to evaluate group differences within the default mode, we established a Bonferroni correction to control for multiple comparisons. Since we explored 11 regions, we divided 0.05 by 11 to obtain a threshold with a *p*-value of 0.0045.

Finally, in order to examine how adaptive behavior and symptom severity related to functional connectivity, we performed a correlational analysis with the functional connectivity contrast vector (obtained by extracting values form a 4mm sphere that surrounded the peak activation of each ROI reported in Table 3.2). The measures that were included were the ADI-R, ADOS and Vineland Adaptive Behavioral Scales (VABS) (Sparrow, Ballo, & Cicchetti, 1984). We determined a priori, several components within the diagnostic measures that were entered into the correlation analysis. For the ADI-R we had "ever" (which includes impairment seen at any point in an individual's life) and "current" (which only codes impairment seen 3 months prior to assessment) codes for the 3 components within the ADI-R. The three components of the ADI-R were: ADI-R social score, ADI-R verbal communication score, ADI-R repetitive and restricted behaviors and interest (RRB) score. There were also 3 similar components for the ADOS, namely the ADOS social, ADOS verbal communication and ADOS RRB score. Finally, we included a measure of overall adaptive functioning obtained from a component of the VABS, known as the VABS composite score. In order to control for multiple comparisons within the correlation analyses, we performed a Bonferroni correction. Since there were 11 regions that showed group differences and a total of 11 subcomponents derived from diagnostic measures, this resulted in a stringent threshold of $p = 0.000413$.

RESULTS

Default network within each group

At a threshold of $p = 0.05$ (family-wise error corrected), both the control group as well as the ASD group showed functional connectivity that was similar to previous reports on the default network in typically developing adult populations (Fox, et al., 2005; Greicius, et al., 2003; Shulman, et al., 1997). Specifically, both groups showed functional connectivity between the PCC seed and regions in the retrosplenial, left/right angular gyrus, left/right medial prefrontal, left/right temporal lobe, left/right superior frontal gyrus, left/right parahippocampal gyrus (refer to Appendix Table 2).

Group differences in the default network

Overall the ASD group showed weaker functional connectivity within the default network relative to controls (refer to Figure 3.1). To assess our first hypothesis that adolescents with ASD would show weaker coupling between the PCC and the right superior frontal gyrus, we performed *t*-tests between groups using the threshold of $p = 0.0045$. Consistent with our hypothesis the control group relative to the ASD group, showed tighter coupling between the PCC and an area of the right superior frontal gyrus $t(22) = 4.02$, $p < 0.0001$, xyz = 28 28 54 (refer to Table 3.2 and Figure 3.2) that was close to coordinates reported in the Monk et al. (*under review*) study. In addition, other clusters within the superior frontal gyrus were also noted (refer to Table 3.2).

To assess our second hypothesis that adolescents with ASD relative to controls would show tighter coupling between the PCC and the right superior temporal gyrus as well as the PCC and right parahippocampal gyrus, we performed a *t*-tests between groups using the threshold of $p = 0.0045$. Consistent with our hypothesis, the ASD group relative

to the control group showed tighter coupling between the PCC and the right superior temporal gyrus $t(22) = 3.69$, $p = 0.001$, xyz = 70 -42 4 (refer to Table 3.2 and Figure 3.3). However, we did not see tighter coupling between the PCC and the right parahippocampal gyrus.

When we assessed our exploratory hypothesis that functional connectivity in the default network in adolescents with ASD would show differences in connectivity than what was previously reported in adults with ASD (Monk, et al., *under review*), we found that similar to the adults with ASD, adolescents with ASD showed tighter coupling between the PCC and the right superior temporal gyrus relative to controls. However, unlike the adult ASD study, we found that weaker connectivity in the ASD group relative to the control group, was not only confined to the coupling between the PCC and the right superior frontal gyrus. Instead, the majority of areas in the default network, with the exception of the right angular gyrus (no group differences) and right superior temporal gyrus, showed weaker coupling in adolescents with ASD relative to controls (refer to Table 3.2 and see Figure 3.4 and Figure 3.5).

To assess the relationship between strength of functional connectivity and measures of adaptive behavior and severity of symptoms within the ASD group, we performed a correlation analysis between the contrast vector for each of the ASD cases and the degree of impairment as measured by the SRS, ADI-R current, ADOS and ADI-R ever as well as the degree of overall adaptive behavior as measured by the VABS (refer to Table 3.3). As described in the methods, we corrected for multiple comparisons because 11 regions and 11 diagnostic subcomponents were tested. Therefore, we divided the p value of 0.05 by 121 to obtain a threshold. At this stringent threshold of $p = 0.000413$, we

found that the connectivity between the PCC and the left angular gyrus (xyz coordinates: -46 -80 30) correlated positively with the VABS composite score (refer to Table 3.3A and Figure 3.6). In addition, no other correlation reached the stringent corrected threshold. To document our findings more fully and to lay the groundwork for future hypothesis, we lowered the threshold to $p = 0.01$. At this threshold, 2 RRB measures (ADI-R current RRB score and the ADOS RRB score) correlated negatively with the strength of connectivity between the PCC and the right superior temporal gyrus (refer to Table 3.3B).

Effects of the level of non-verbal cognitive functioning

In our sample, the Vineland adaptive behavioral scale (VABS) score correlated with the level of non-verbal cognitive functioning (r=0.672, p=0.023). In order to further assess the relationship between the VABS and the strength of connectivity between the PCC and the left angular gyrus, we carried out a follow-up linear regression with non-verbal cognitive functioning as a covariate. As expected, the VABS was a significant predictor for strength of connectivity between the PCC and the left angular gyrus, β=19.06, t=4.55, p=0.002. On the other hand, within the multiple linear regression framework, the non-verbal cognitive functioning score did not predict strength of connectivity between the PCC and the left angular gyrus, β =0.19, t=0.04, p=0.97. However, it should be noted that because there is considerable overlap between these two measures, a multiple regression analysis might not be able to accurately tease apart the variances that would be attributed to each of these measures.

Effects of medication

In order to assess whether medications played a role in influencing our results, we followed a previous study by Kennedy and Courchesne (2008) on resting connectivity in

adults with ASD and excluded the 7 adolescents with ASD who were on medications. At a threshold of $p = 0.05$ (uncorrected), the ASD group, which comprised of 5 remaining adolescents with ASD who were not on medications, continued to show functional connectivity within the default network, between the PCC seed and regions in the retrosplenial, left/right angular gyrus, left/right medial prefrontal, left/right temporal lobe, left/right superior frontal gyrus, left/right parahippocampal gyrus.

In addition, when the remaining 5 adolescents with ASD were compared against the controls, we saw a similar pattern of results at a threshold of $p = 0.05$ (uncorrected). First, the control group relative to the ASD group continued to show tighter coupling between the PCC and the right superior frontal gyrus $t(15) = 2.64$, $p = 0.009$, xyz = 28 28 54. Second, the ASD group relative to the control group continued to show tighter coupling between the PCC and the right superior temporal gyrus $t(15) = 2.40$, $p = 0.015$, xyz = 70 -42 4. Finally, the same patterns of weaker connectivity between the PCC and areas of the default network were found in this subsample of adolescents with ASD.

DISCUSSION

In this study, we examined functional connectivity of the default network in the absence of a cognitive task in adolescents with ASD and controls. When we compared the functional connectivity between these groups, the ASD relative to the control group showed both weaker and tighter coupling. First, as predicted, the ASD group relative to the control group showed weaker coupling between the PCC and the right superior frontal gyrus. Second, the ASD group relative to the control group showed tighter coupling between the PCC and the right superior temporal gyrus but not between the PCC and the right parahippocampal gyrus. Third, when the findings in this present

chapter were compared to a prior study in our lab that examined connectivity within the default network in a sample of adults with ASD (Monk, et al., *under review*), we found that in the adolescent sample, the majority of pairwise couplings between the PCC and other areas of the default network showed weaker connectivity in adolescents with ASD relative to controls than what was previously reported in the adult sample. Finally, when we examined how diagnostic measures within the ASD group correlated with strength of connectivity within areas of the default network, we found that the VABS, which gives an indication of the child's adaptive behavior, showed a strong positive correlation with the connectivity between the PCC and left angular gyrus.

Functional connectivity and developmental differences

Consistent with the adult ASD study by Monk et al., (*under review*), we found weaker coupling between the PCC seed and the right superior frontal gyrus in adolescents with ASD relative to controls. In addition, we found tighter coupling between the PCC seed and the right superior temporal gyrus in adolescents with ASD relative to controls. Unlike the Monk et al., (*under review*) study, we did not find a similar pattern of tighter coupling between the PCC seed and the right parahippocampal gyrus in adolescents with ASD relative to controls. Instead, we found that the majority of the pairwise couplings showed weaker functional connectivity within the default network in adolescents with ASD. When we compared the findings in this present chapter to two other resting connectivity studies in ASD, we found several areas of similarity and differences. In a study by Kennedy and Courchesne (2008), although they selected a different seed and their findings were more region specific, they too found reduced connectivity in the default network in adults with ASD relative to controls (Kennedy & Courchesne, 2008).

These differences were least pronounced in the right superior temporal areas but most pronounced in the medial prefrontal areas and left angular gyrus (Kennedy & Courchesne, 2008). The findings of the second study by Cherkassky et al., (2006) lend even more support to our findings of reduced connectivity. In this study, they reported up to 94% of the pairwise couplings showing weaker connectivity in adults with ASD relative to controls. However, while the Cherkassky et al., (2006) paper lends support to our findings, the resting connectivity data was collected over several blocks of very short durations instead of a single 10-minute session.

Even though the resting connectivity data presented in this chapter was collected and analyzed in the same way as the Monk et al. *(under review)* study, the mean age of the adult ASD sample was 26 (ranging from 19-37) years of age. On the other hand, the mean age of the adolescent ASD sample included in this study was approximately 15 (ranging from 13-17) years of age. Clearly, the differences in ages between studies could account for the discrepancies in results across studies.

Indeed, adolescence is a period where rapid gains in cognition take place (Casey, Giedd, & Thomas, 2000) and many studies have reported age related changes within the brain that are most pronounced during adolescence (Giedd, 2008). DTI studies measuring white matter integrity have reported evidence for greater efficiency of neuronal communication throughout adolescence (Cascio, Gerig, & Piven, 2007). In addition, studies have emphasized that the density of gray matter in the adolescent brain differs from the adult brain (Sowell, Thompson, Tessner, & Toga, 2001). These differences are most pronounced in the frontal and striatal regions and have been suggested to coincide with increased levels of cognitive functioning during adulthood (Reiss, Abrams, Singer,

Ross, & Denckla, 1996). For example, evidence from functional imaging studies have reported greater activation in adults as compared to adolescence and children at younger ages in brain regions associated with language and communication (Booth, et al., 2001; Turkeltaub, Gareau, Flowers, Zeffiro, & Eden, 2003).

In the default network, there has been evidence for age-related changes in normative development (Fair, et al., 2008). Specifically, Fair et al., (2008) found weaker connectivity within the default network in younger children, ages 7-9 years old when they compared their results with other studies of the default network in typically developing adults. Although they used a seed that was in the ventromedial prefrontal cortex and their sample involved younger children, their finding seems to be consistent with the pattern that we found in our sample of adolescents.

Visual inspection of the adult resting connectivity data by Monk et al. *(under review)* and the adolescent data presented in this chapter, suggest that the adult and adolescent ASD samples showed similar connectivity within the default network, but the adolescent controls showed stronger connectivity than the adult controls. This accounts for the differing findings between the present adolescent study and the Monk et al., *(under review)* study involving adults. Moreover, as described above, the study that examined the default network in children (ages ranged from 7-9 years old) reported that connectivity was weaker than adults (Fair, et al., 2008). Thus, in normative development, adolescence may be a period of heightened connectivity relative to earlier and later developmental stages. Further analyses across studies are necessary to verify this suggestion and to better understand the relevance of this finding to adolescents with ASD

Correlation between diagnostic measures and strength of connectivity

To our knowledge, no fMRI study has focused on examining the brain correlates of adaptive functioning in individuals with ASD. Adaptive functioning as assessed by the VABS provides an index for measuring an individual's independence and ability to cope with the demands of every day life. It includes aspects of communication and socialization as well as how adept he/she is at responding to changes in the environment (Liss, et al., 2001; Sparrow, et al., 1984). It is a crucial area of study since there have been reports that even high functioning individuals with ASD, show deficits in adaptive behavior despite normal IQ ranges (Klin, et al., 2007). In addition, better adaptive behavioral skills in ASD can result in better outcomes later in life (Mazefsky, Williams, & Minshew, 2008). The strong positive correlation that we found in the ASD group between the VABS and strength of connectivity/coupling between the PCC and left angular gyrus, suggests that better synchrony within the default network might relate to better adaptive behavior. Future research should focus on the various functions that the default network subserves. This would enable better characterization of the role that the default network plays in psychopathology.

In order to document our findings and lay the groundwork for future hypothesis, at a more liberal threshold of $p < 0.01$, we reported that RRB scores as assessed by the ADI-R and ADOS consistently showed a negative correlation with connectivity within the right superior temporal gyrus (refer to Table 3.3B). This suggests that increased impairment in the RRB domain is associated with decreased connectivity within the right temporal lobe. This is consistent with a fMRI study which reported that adolescents with ASD showed that more severe RRBs were associated with hypoactivation within the temporal regions (Freitag, et al., 2008). In contrast, the Monk et al. *(under review)* adult

ASD study showed that RRBs were associated with functional connectivity with another region of the default network, namely, the parahippocampal gyrus. It is interesting to note that not only was this association in a different region then what we report here but the pattern was also reversed whereby, more severe RRBs were associated with increased connectivity. This suggests that different brain regions might assert varying influence on the RRB phenotype at different life stages.

Limitations

There are several limitations to the study. First, because the resting connectivity protocol consists of one functional run that lasted for 10 minutes, the data could not be averaged and this made it susceptible to noise as well as motion and physiological artifacts. However, in addition to performing physiological correction on the data, we covaried out the 6 head motion parameters in our regression analysis. This enabled us to obtain a clearer signal. Second, because this procedure does not require the participant to engage in a specific cognitive task, but instead, measures functional connectivity during resting state, it is not possible to investigate what thought processes underlie differences in intrinsic connectivity between adolescents with ASD and controls. In addition, we were unable to control for attention differences between groups that might have occurred during the time that the default network was monitored. However, studies in which data were acquired during sleep and anesthesia, processes that are below the level of conscious awareness, also reported that the brain engages a similar network (Horovitz, et al., 2008; Kiviniemi, et al., 2000). Thus it is unlikely that group differences reported here are due to spontaneous thought processes but instead reflect differences in the brains intrinsic connectivity. Finally, since we only explored resting connectivity in 11 regions,

this might have limited the scope of our study and prevented us from exploring other regions that showed group differences outside the default network.

Implications and future directions

As mentioned above, future work is needed to examine if adolescence in normative development is marked by magnified differences in functional connectivity as compared to pre- and post adolescent periods. In addition, future studies should tap younger age ranges and include lower functioning individuals with ASD so as to obtain a more holistic picture of intrinsic connectivity. This will help us to clarify how connectivity relates to adaptive functioning and symptoms severity. Lastly, studies examining the distribution, density and properties of white matter tracts between regions in the default network through diffusion tensor imaging (DTI) techniques will help to elucidate the anatomical bases of the abnormal functional connectivity.

Conclusions

To our knowledge this is the first study to explore intrinsic connectivity within the default network in adolescents with ASD. These findings extend previous reports of abnormal patterns of intrinsic connectivity in ASD when participants are not engaged in a cognitive task. In this Chapter, we reported weaker functional connectivity within the default network in adolescents with ASD, with the exception of the right superior temporal gyrus. This pattern of results appear to be associated with changes in the default network during adolescence. Moreover our findings suggest that increased coherence within the default network is related to higher levels of adaptive behavior in adolescents with ASD.

TABLES

Table 3.1: Subject Characteristics

	ASD	**Control**	***t* (df)**	***p* value**
Age, mean (SD)	15 (1.44)	15 (1.51)	1.29 (22)	0.21
Age range	13 - 17	13 - 18		
Male to female ratio	11:01	11:01		
Verbal cognitive functioning, mean (SD)	114 (18.26)	117 (12.43)	0.33 (22)	0.75
Nonverbal cognitive functioning mean (SD)	116 (11.67)	108 (11.05)	1.78 (22)	0.09
Handedness left to right ratio	1:11	1:11		

Table 3.2: Functional connectivity between the PCC and other regions of the default network where group differences were found. Threshold was set at $p = 0.0045$ based on a Bonferroni correction. The ASD group showed widespread weaker coupling in the default network relative to the control group. The ASD group showed tighter coupling in the superior temporal gyrus. Only major clusters were reported. **Note:** Both the ASD and control groups alone showed robust activation in the default network (refer to Appendix Table 2).

Region	Comparison	Cluster size (k)		MNI coordinates		
			t	x	y	z
Retrosplenial	ASD-Controls		ns			
	Controls-ASD	11	3.41	8	-54	16
		8	3.15	6	-54	4
		1	3.03	-18	-42	-4
		1	2.97	-16	-46	0
Left angular gyrus	ASD-Controls		ns			
	Controls-ASD	13	3.2	-46	-80	30
Right angular gyrus	ASD-Controls		ns			
	Controls-ASD		ns			
Left medial prefrontal	ASD-Controls		ns			
	Controls-ASD	42	3.78	-28	64	12
		57	3.71	-12	52	-2
		26	3.67	-22	60	2
		16	3.25	-14	44	12
Right medial prefrontal	ASD-Controls		ns			
	Controls-ASD	3	3.08	26	52	-4
		3	3.04	24	56	-6
		4	2.98	8	68	-2
		2	2.96	18	24	42
		1	2.87	2	44	12
Left temporal lobe	ASD-Controls		ns			
	Controls-ASD	22	4	-20	-44	-2
		75	4	-40	4	-16
		12	3.97	-36	-42	-10
		10	3.88	-36	-34	-24
		17	3.56	-58	-74	16
		23	3.55	-60	-72	10
		23	3.45	-26	-42	-16
		19	3.31	-48	-36	8
		26	3.25	-48	-80	28
		3	3.24	-42	-28	-24
		7	3.21	-48	8	-40
		1	2.91	-36	14	-30

Right temporal lobe	ASD-Controls	3	3.69	70	-42	4
	Controls-ASD	1342	5.36	40	-54	16
		41	4.45	34	-32	16
		28	3.71	44	-20	8
		16	3.28	40	-2	-36
		2	3.05	18	-54	16
		1	2.9	36	-32	-20
Left superior frontal gyrus	ASD-Controls		ns			
	Controls-ASD	95	4.14	-20	58	2
		94	4.06	-12	-16	72
		24	3.64	-24	70	0
		31	3.36	-18	56	-12
		5	3.27	-28	-4	68
		2	3.08	-26	64	14
		2	2.95	-10	4	66
		2	2.93	-30	24	54
		1	2.9	-26	44	-16
Right superior frontal gyrus	ASD-Controls		ns			
	Controls-ASD	16	4.02	28	28	54
		56	3.85	22	-14	68
		78	3.63	10	30	52
		5	3.62	4	44	52
		29	3.49	24	54	-4
		12	3.12	22	64	-12
		2	3.05	8	48	46
		3	3.04	12	4	66
		1	2.87	8	68	-4
Left parahipocampal gyrus	ASD-Controls		ns			
	Controls-ASD	48	5.04	-16	0	-16
		31	4.7	-36	-32	-24
		217	4.35	-34	-42	-10
		2	3.2	-10	-36	-2
		7	3.15	-28	-60	-8
Right parahipocampal gyrus	ASD-Controls		ns			
	Controls-ASD	171	4.16	18	-14	-22

Table 3.3: Correlations between symptom severity scores and functional connectivity between the PCC and each of the other areas of the default mode in adolescents with ASD. **Table 3.3A:** Reports correlations with regions that showed tighter coupling in the control group relative to the ASD group. **Table 3.3B:** Reports correlations with regions that showed tighter coupling in the ASD group relative to the control group. **Legend**: diagnostic measures: SRS = social responsiveness scale, ADI-R = Autism Diagnostic Interview-Revised. ADOS = Autism Diagnostic Observation Schedule. VABS = Vineland adaptive behavioral composite scores. **Note:** 1) ADI-R "current" scores code symptoms seen within past 3 months prior to clinical interview. ADI-R "ever" score codes symptoms throughout the individual's life. 2) One of the 12 ASD subjects did not have Vineland scores. 3) Higher scores on the SRS, ADI-R and ADOS reflect more severe symptoms. For the VABS, higher scores reflect better overall adaptive behavioral functioning. * $p = 0.05$ (two-tailed) and ** $p = 0.01$ (two tailed).

A.

Default Regions		SRS	ADI social current	ADI verbal comm current	ADI RRB current	ADOS social	ADOS comm	ADOS RRB	ADI social ever	ADI verbal comm ever	ADI RRB ever	VABS
Retrosplenial	Pearson Correlation	.598*	-0.031	0.255	-0.341	-0.393	-0.508	-0.174	-0.264	0.229	-0.261	0.306
	Sig. (2-tailed)	.040	0.923	0.424	0.278	0.206	0.092	0.588	0.407	0.474	0.413	0.360
	N	12	12	12	12	12	12	12	12	12	12	11
Left angular gyrus	Pearson Correlation	.091	-0.460	-0.226	-0.459	-0.458	-0.377	-0.285	-.628*	-0.373	-0.417	.909**
	Sig. (2-tailed)	.779	0.132	0.480	0.133	0.134	0.227	0.370	0.029	0.233	0.177	0.000
	N	12	12	12	12	12	12	12	12	12	12	11
Left medial prefrontal	Pearson Correlation	.553	0.098	0.079	-0.477	-0.062	-0.176	-0.566	-0.151	0.228	-0.348	0.593
	Sig. (2-tailed)	.062	0.761	0.807	0.117	0.849	0.584	0.055	0.639	0.477	0.268	0.054
	N	12	12	12	12	12	12	12	12	12	12	11
Right medial prefrontal	Pearson Correlation	.251	-0.377	-0.307	-0.148	-0.197	-0.126	0.085	-0.175	-0.173	-0.038	-0.036
	Sig. (2-tailed)	.432	0.227	0.332	0.646	0.539	0.697	0.793	0.586	0.591	0.906	0.917
	N	12	12	12	12	12	12	12	12	12	12	11

Default Regions		SRS	ADI social current	ADI verbal comm current	ADI RRB current	ADOS social	ADOS comm	ADOS RRB	ADI social ever	ADI verbal comm ever	ADI RRB ever	VABS
Left temporal lobe	Pearson Correlation	-.082	0.052	0.220	-0.240	-0.052	-0.231	-0.192	-0.369	0.141	-0.282	0.067
	Sig. (2-tailed)	.799	0.872	0.492	0.452	0.871	0.471	0.550	0.238	0.661	0.374	0.846
	N	12	12	12	12	12	12	12	12	12	12	11
Right temporal lobe	Pearson Correlation	.378	0.031	0.222	-0.531	-0.058	-0.024	-0.543	-0.349	0.394	-.660*	0.271
	Sig. (2-tailed)	.226	0.925	0.487	0.076	0.858	0.942	0.068	0.266	0.205	0.020	0.420
	N	12	12	12	12	12	12	12	12	12	12	11
Left superior frontal gyrus	Pearson Correlation	.141	0.093	0.199	-0.414	-0.137	-0.193	-0.413	-0.281	-0.053	-0.488	0.473
	Sig. (2-tailed)	.661	0.774	0.534	0.180	0.672	0.548	0.182	0.377	0.869	0.107	0.142
	N	12	12	12	12	12	12	12	12	12	12	11
Right superior frontal gyrus	Pearson Correlation	.362	0.246	0.549	-0.221	0.253	0.261	-0.517	-0.151	0.537	-0.282	0.098
	Sig. (2-tailed)	.248	0.442	0.065	0.490	0.427	0.412	0.085	0.639	0.072	0.374	0.775
	N	12	12	12	12	12	12	12	12	12	12	11
Left parahippocampal gyrus	Pearson Correlation	.201	-0.169	0.091	-0.325	0.075	-0.210	-0.306	-0.166	0.349	-0.249	0.155
	Sig. (2-tailed)	.532	0.600	0.777	0.303	0.817	0.511	0.334	0.606	0.266	0.435	0.648
	N	12	12	12	12	12	12	12	12	12	12	11
Right parahippocampal gyrus	Pearson Correlation	.044	0.369	0.324	0.154	0.394	0.446	-0.146	0.183	.676*	-0.024	-0.104
	Sig. (2-tailed)	.891	0.239	0.305	0.633	0.205	0.147	0.652	0.569	0.016	0.942	0.761
	N	12	12	12	12	12	12	12	12	12	12	11

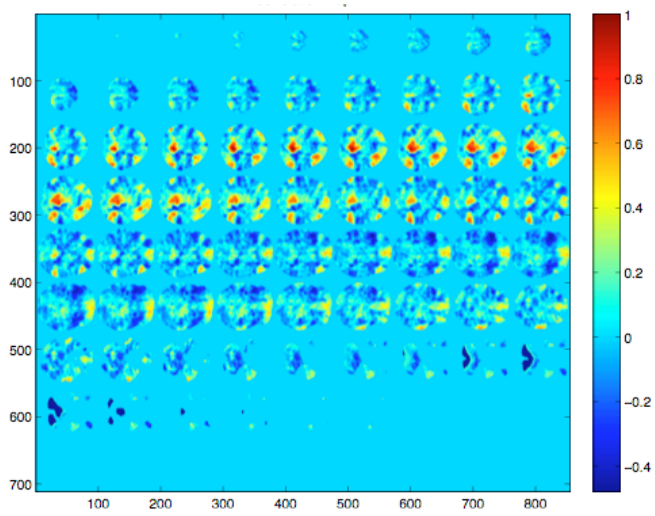
B.

Default Regions		SRS	ADI social current	ADI verbal comm current	ADI RRB current	ADOS social	ADOS comm	ADOS RRB	ADI social ever	ADI verbal comm ever	ADI RRB ever	VABS
Right superior temporal gyrus	Pearson Correlation	.418	.383	.019	-.717**	-.123	-.199	-.742**	-.314	.113	-.688*	.368
	Sig. (2-tailed)	.176	.219	.952	.009	.704	.536	.006	.320	.726	.013	.266
	N	12	12	12	12	12	12	12	12	12	12	11

FIGURES

Figure 3.1: Connectivity map of an ASD case and a control case. The maps are generated by computing the correlations between the PCC (seed region: MNI xyz coordinate: -5 -53 41) with other areas of the default mode. **Fig 3.1A:** ASD case showing reduced connectivity in the default network. **Fig 3.1B:** Control case shows a clear demarcation of the default network.

A.



B.

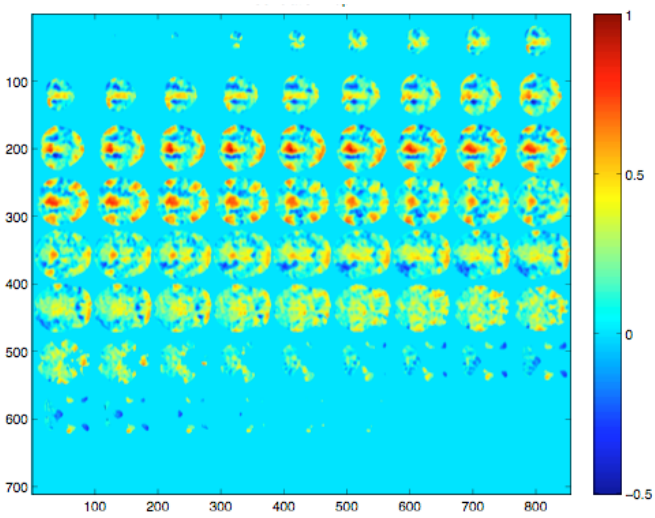


Figure 3.2: The control group showed tighter coupling within the right superior frontal gyrus relative to the ASD group $t(22) = 4.02$ $p < 0.001$, xyz = 28 28 54. For illustration purposes, the threshold was set at $p < 0.005$ with a cluster size of $k > 100$ voxels.

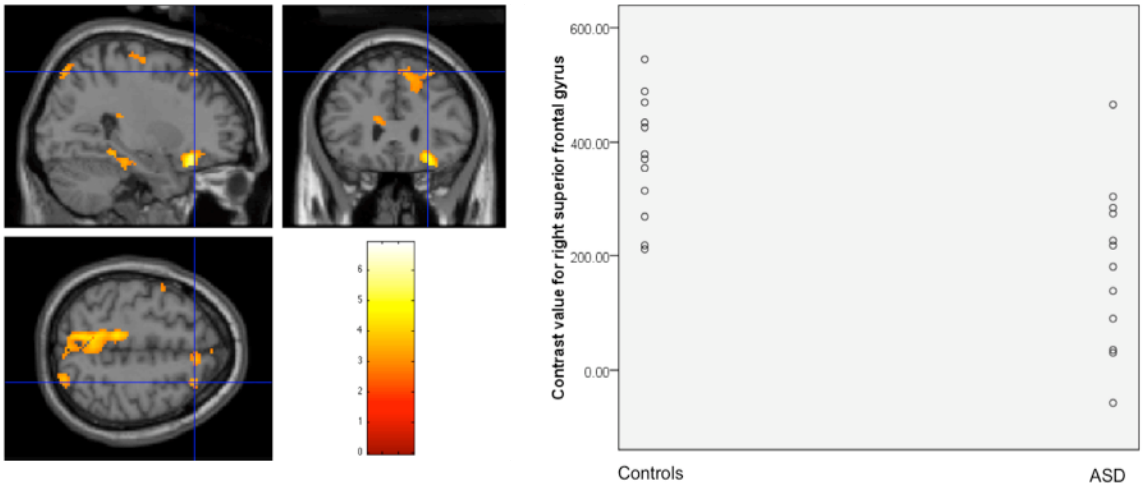


Figure 3.3: The ASD group showed tighter coupling within the right superior temporal gyrus relative to the control group $t(22) = 3.69$ $p = 0.001$, xyz = 70 -42 4. For illustration purposes, the threshold was set at $p < 0.05$.

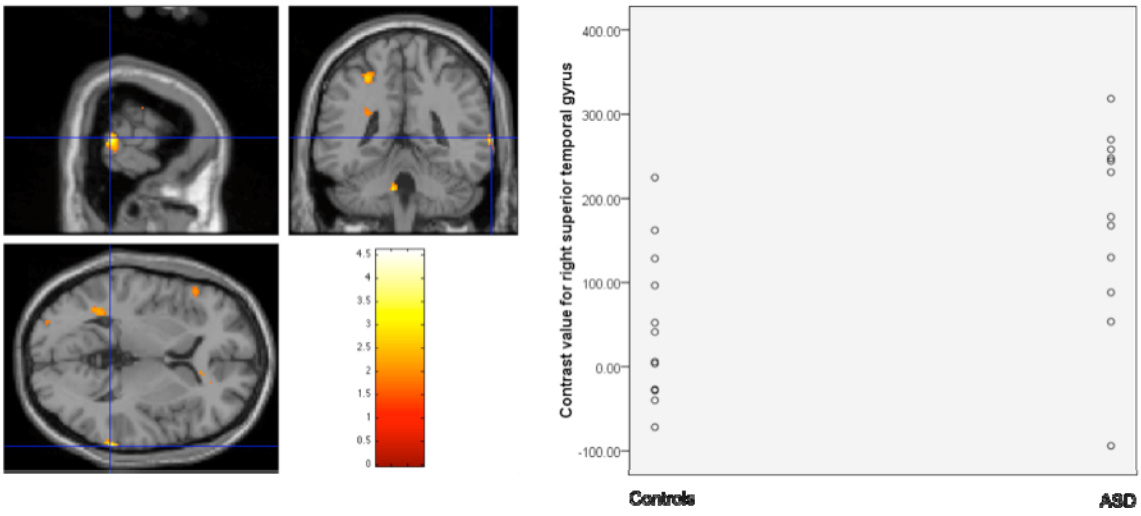


Figure 3.4: The control group showed tighter coupling within the right parahippocampal gyrus relative to the ASD group $t(22) = 4.16$ $p < 0.001$, xyz = 18 -14 -22. For illustration purposes, the threshold was set at $p < 0.005$ with a cluster size of $k > 100$ voxels.

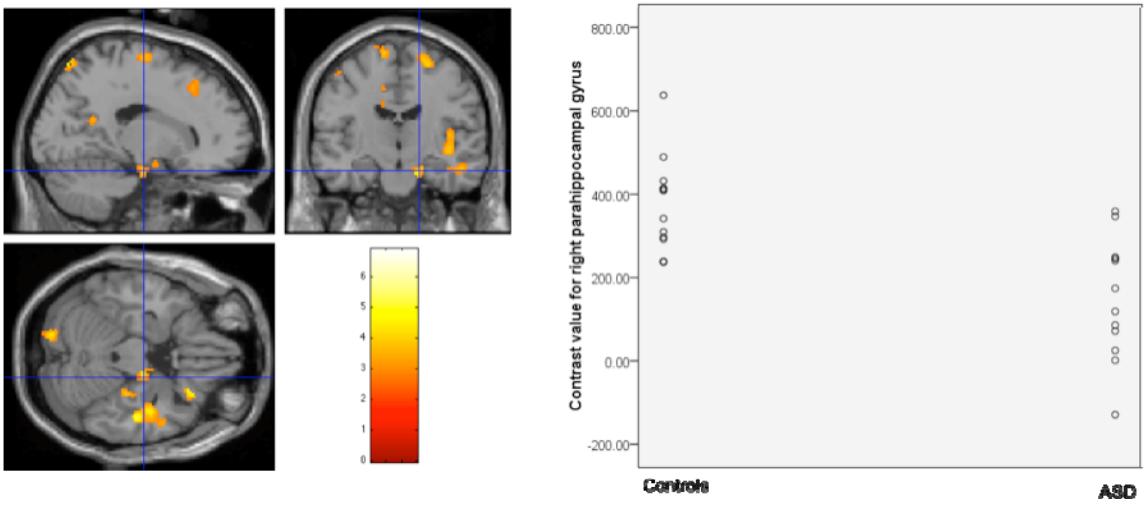


Figure 3.5: The control group showed tighter coupling within the retrosplenial region relative to the ASD group $t(22) = 3.41$ $p = 0.001$, xyz = 8 -54 16. For illustration purposes, the threshold was set at $p < 0.005$ with a cluster size of $k > 100$ voxels.

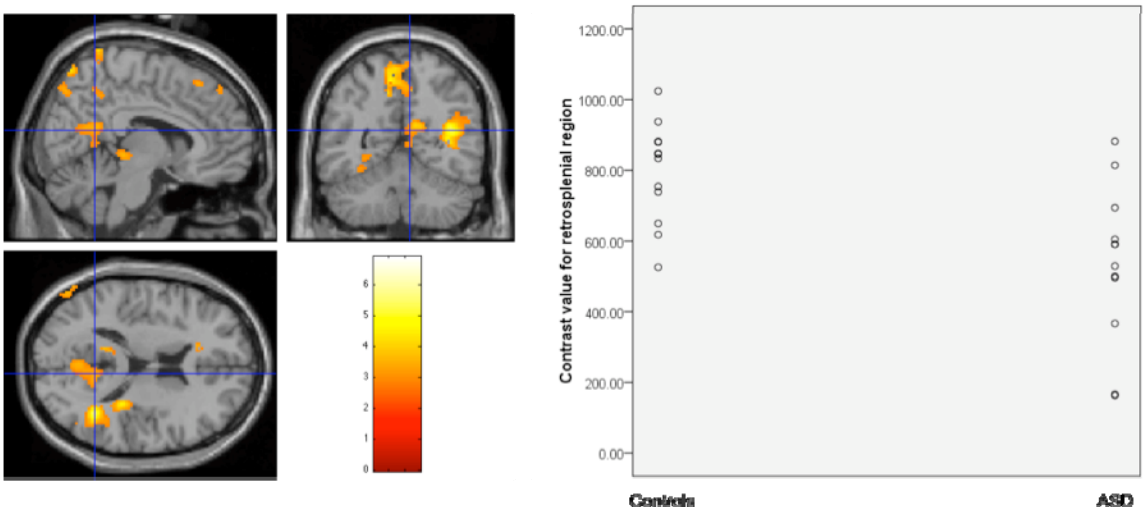
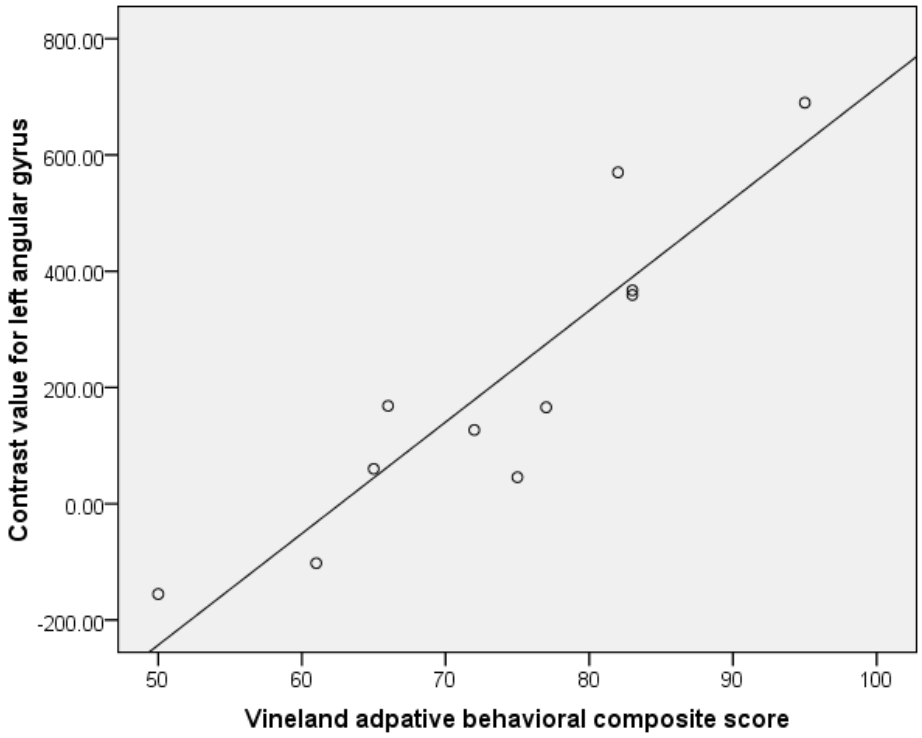


Figure 3.6: Within the ASD group, overall adaptive behavior as measured by the VABS composite score, was positively correlated with functional connectivity within the left angular gyrus. Contrast values were extracted from a 4mm sphere surrounding the peak activation $t(11) = 3.2$, xyz = -46 -80 30 and plotted with the VABS composite score, Pearson $r = 0.909$, $p = 0.000104$. **Note:** Higher scores on the VABS indicate better overall adaptive functioning.



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CHAPTER IV

CONCLUSIONS

The set of studies presented here in this dissertation are the first to show evidence for an alteration in functional connectivity within the neural networks of adolescents with ASD relative to controls and that the strength of connectivity related to specific core impairments of ASD. In Chapter II, functional connectivity between the amygdala and cortical structures was examined in the presence of a social task with emotional faces. Although the behavioral data did not show differences in performance between groups, the PPI analysis revealed that relative to controls, adolescents with ASD showed less positive connectivity/coupling between the amygdala and the right middle temporal gyrus, when viewing emotional faces versus baseline. Additionally, relative to controls, adolescents with ASD showed less positive coupling between the amygdala and the inferior frontal gyrus, areas that have been identified to be play a role in empathy. Interestingly, the findings within the inferior frontal gyrus were specific to sad versus baseline conditions, suggesting that empathy provoking stimuli may have less of an impact on a subpopulation of adolescents with ASD. Moreover, we found that adolescents with greater social impairments were associated with less positive connectivity within the right middle temporal lobe but greater positive connectivity within the inferior frontal gyrus.

In Chapter III, we examined intrinsic/resting-state connectivity within the default

network in an overlapping sample of adolescents with ASD in the absence of a cognitive task. Relative to controls, we found weaker connectivity in the majority of pairwise couplings within the default network in adolescents with ASD. However, we also found evidence for one region, the right superior temporal gyrus, which showed tighter coupling with the posterior cingulate cortex seed in adolescents with ASD relative to controls. Moreover, within the ASD group, poorer adaptive behavior was associated with weaker coupling between the posterior cingulate cortex seed and the left angular gyrus. This suggests that less synchrony within the default network might underlie deficits in ASD. In sum, the results of Chapter II and Chapter III are complementary in that both report an overall pattern of weaker functional connectivity in adolescents with ASD relative to controls and that these alterations in connectivity within adolescents with ASD are associated with severity of symptoms. The findings in this adolescent sample are consistent with prior reports of weaker functional connectivity in adults with ASD (Just, Cherkassky, Keller, Kana, & Minshew, 2007; Koshino, et al., 2008; Turner, Frost, Linsenbardt, McIlroy, & Muller, 2006; Villalobos, Mizuno, Dahl, Kemmotsu, & Muller, 2005; Welchew, et al., 2005). In addition, it extends the current literature, which documenting disturbances in functional connectivity, to younger age ranges.

Investigating functional connectivity in ASD is a complement to other methodologies. As outlined in Chapter I, histological methods involving post mortem brain tissue as well as genetic analysis using animal models, have contributed to our understanding of abnormalities in cortical organization as well as neural development in ASD. However, both these methods have their limitations. For example, not only is human brain tissue difficult to acquire, but the tissue obtained from each individual case

can sometimes be stored in different types of fixative for varying amounts of time. Specifically, post mortem intervals (time elapsed since death) as well as cause of death can vary from case to case. All these factors contribute to the heterogeneity within ASD samples (Persico & Bourgeron, 2006). Genetic analysis using animal models has also proved to be challenging. Although knock out mice as well as other genetic manipulations in animal research has enabled us to understand how genes can alter neural development, an accurate animal model of autism, which captures deficits in all three domains has yet to be established. Unlike post mortem studies and animal models, functional MRI methods allow us to characterize abnormalities in brain connectivity in living individuals with ASD. In addition, this enables us to isolate and examine the functional connectivity between brain structures that are involved in higher order cognitive processes as well as during resting-state and allows us to relate these disruptions in functional connectivity to specific behavioral deficits that are present in individuals with ASD. Though each methodological approach has its limitations, together, results obtained from these various techniques can contribute to our knowledge of uncovering alterations in brain connectivity in ASD. Indeed, there is an emergent consensus across these various approaches that suggest that ASD may be a disorder of connectivity (Belmonte, et al., 2004; Geschwind & Levitt, 2007).

In this dissertation, we report widespread weaker connectivity with some areas showing tighter functional connectivity in adolescents with ASD relative to controls. This not only provides support that brain connectivity is altered in our sample of adolescents with ASD but also sheds light on possible genetic variations/ mutations that might give rise to altered functional connectivity within this population. More specifically, our

findings of widespread weaker connectivity lend support to studies that have implicated susceptibility genes such as the RELN gene variant that are involved in abnormal cell migration during pre-natal development in ASD (Fatemi, et al., 2005). In addition, our pattern of tighter connectivity between the PCC and the superior temporal gyrus might be a result of disruptions during the process of synaptogenesis. Because the default network has been known to be involved in maintaining the homeostasis of excitatory and inhibitory neuronal inputs (Biswal, Yetkin, Haughton, & Hyde, 1995), our findings of altered connectivity in the default network in adolescents with ASD are consistent with studies that have implicated Neuroligin genes which are involved in maintaining the balance between excitatory and inhibitory inputs. Indeed, there have been reports that there are mutations within the Neuroligin 3 and Neuroligin 4 gene in individuals with ASD (Jamain, et al., 2003). In sum, these findings suggest that altered functional connectivity could be a result of variations and/or mutations within specific genes that play a role in neural development in ASD. Finally, research in this area can lay the groundwork for elucidating the precipitating events that occur during pre- and post-natal development and can help to delineate specific subtypes within ASD.

Additional work should be carried out in order to examine changes in functional connectivity that take place during normative development. There is a great need for better characterization of how functional connectivity between specific brain regions change during periods where others have reported changes in brain structure as well as grey and white matter concentration throughout the brain (Giedd, et al., 1996; Sowell, et al., 2003). Such information will be able to guide future studies in uncovering the neural mechanisms that underlie disorders such as ASD.

Similarly, ASD research would benefit from studies documenting changes in functional connectivity through development. Although others have sought to chart structural changes within specific brain regions with increasing age (Schumann, et al., 2004; Sparks, et al., 2002), our study is the first to document disruptions in functional connectivity during earlier stages of development. Studies would benefit from adopting a developmental perspective to examining functional connectivity. Moreover, because intrinsic connectivity has been reported to occur in the absence of a cognitive task and even during sleep, future studies could aim to uncover changes in functional connectivity within the default network in younger children with ASD as well as infant siblings of children with ASD (at-risk individuals). Similarly, since there are no cognitive demands associated with a task in such protocols, it will also be possible to examine functional connectivity within the default network in lower functioning individuals with ASD. Incorporating individuals at younger ages as well as across various functioning levels would enable us to gain a more holistic understanding of functional connectivity throughout development as well as throughout the autism spectrum.

In addition, follow-up studies using diffusion tensor imaging (DTI) can be used to clarify or guide future research on functional connectivity. This would allow us to examine whether individuals with ASD show evidence for changes in white matter integrity within regions that show altered functional connectivity. Finally, it will be worthwhile to explore how alterations in functional connectivity relate directly to variations within susceptibility genes. More information is needed to understand how the brain mediates genetic polymorphisms to yield phenotypic differences. The findings presented in this dissertation provide initial evidence for altered functional connectivity

in a sample of adolescents with ASD and how this altered connectivity relates to severity of social symptoms and adaptive behavior. These findings are an initial step in laying the groundwork for future research of functional connectivity within younger individuals with ASD.

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APPENDIX

Table1: Whole brain table showing areas of activation in response to emotional faces versus baseline. Threshold was set at p = 0.001 and only clusters of k > 10 were reported.

Group	*t*	Cluster size	Location	MNI Coordinates		
				X	Y	Z
Fearful vs Neutral						
Controls	4.79	21	postcentral gyrus	-58	-30	48
ASD	N.S					
ASD-Controls	N.S					
Controls-ASD	4.54	37	postcentral gyrus	-10	-40	66
	4.15	14	medial frontal gyrus	18	-20	60
	3.97	43	anterior cingulate	-10	22	-4
	3.91	24	inferior parietal lobe	-58	-42	26
Happy vs Neutral						
Controls	4.18	25	postcentral gyrus	40	-30	34
	3.75	13	middle temporal gyrus	42	-44	0
ASD	4.26	19	cingulate gyrus	18	-12	38
	4.24	16	middle occipital gyrus	30	-94	-6
ASD-Controls	N.S					
Controls-ASD	N.S					
Sad vs Neutral						
Controls	3.91	16	temporal lobe	40	-48	2
ASD	4.78	50	inferior parietal lobe	-42	-50	50
	4.17	101	postcentral gyrus	38	-44	62
	3.99	20	putamen	-28	-26	0
	3.87	13	superior temporal gyrus	38	4	-18
	3.78	15	middle temporal gyrus	-44	-54	-2
ASD-Controls	4.02	16	thalamus	-26	-24	0
	3.87	55	postcentral gyrus	34	-48	64
Controls-ASD	4.36	51	anterior cingulate	-14	30	-6
	3.93	24	anterior cingulate	12	36	26
	3.84	13	insula	36	20	6
Fearful vs Baseline						
Controls	12.18	50258	lingual gyrus	26	-60	-4

	5.3	283	precentral gyrus	-6	-22	74
	4.02	66	middle frontal gyrus	-40	46	26
ASD	8.18	6198	lingual gyrus	28	-62	-4
	6.17	297	inferior frontal gyrus	-56	12	34
	5.54	770	thalamus	-14	-10	4
	5.24	81	parahippocampal gyrus	30	-16	-14
	5.22	235	inferior frontal gyrus	50	28	18
	4.79	126	superior frontal gyrus	-8	10	66
	4.71	154	superior temporal grus	60	-44	8
	4.56	28	postcentral gyrus	-52	-24	56
	4.28	125	inferior frontal gyrus	52	2	34
	4.28	25	left fusiform	-42	-58	-14
	4.23	24	thalamus	16	-14	-2
	4.19	192	postcentral gyrus	-42	-24	42
	3.98	91	inferior frontal gyrus	52	-48	42
	3.96	36	medial frontal gyrus	4	42	30
	3.95	41	middle frontal gyrus	38	18	28
	3.7	28	insula	-42	6	-4
	3.87	10	cuneus	16	-98	18
	3.83	12	superior temporal grus	42	10	-12
ASD-Controls	N.S					
Controls-ASD	N.S					
Happy vs Baseline						
Controls	12.13	48322	posterior cingulate	20	-68	10
	4.77	256	postcentral gyrus	60	-14	26
	4.39	46	superior frontal gyrus	26	62	-8
	4.09	21	cingulate gyrus	24	-12	40
	3.86	39	middle temporal gyrus	50	-18	-14
	3.85	13	orbital gyrus	10	50	-20
ASD	9.5	13587	occipital lobe area	30	-62	-4
	6.23	1543	substania nigra	-14	-22	6
	5.77	205	superior temporal gyrus	-54	-12	8
	5.7	1528	lentiform nucleus	12	4	-2
	4.5	32	inferior frontal gyrus	60	4	22
	4.49	63	precentral gyrus	-58	0	34
	4.46	25	middle frontal gyrus	50	36	26
	4.39	191	inferior frontal gyrus	40	28	12
	4.38	62	fusiform gyrus	-44	-54	-18

	4.34	178	precentral gyrus	-54	-18	38
	4.29	57	superior frontal gyrus	22	48	-16
	4.29	37	superior temporal gyrus	44	-12	-12
	4.25	11	lentiform nucleus	26	-16	-6
	4.13	21	supramarginal gyrus	42	-50	28
	4.11	15	superior temporal gyrus	48	6	-4
	4.03	25	inferior parietal lobule	52	-36	44
	4	76	cingulate gyrus	-4	20	30
	3.95	69	middle frontal gyrus	-20	38	-10
	3.94	19	superior temporal gyrus	-58	-42	6
	3.74	17	medial frontal gyrus	4	46	30
	3.69	14	inferior frontal gyrus	38	8	28
	3.68	18	postcentral gyrus	60	-20	14
ASD-Controls	4.77	49	middle occipital gyrus	52	-75	0
	4.75	117	lingual gyrus	32	-62	-4
	4.17	36	middle temporal gyrus	50	-52	-6
	4.14	43	cingulate gyrus	14	-8	40
	4.12	43	middle occipital gyrus	-30	-74	0
	3.97	16	middle frontal gyrus	-22	38	-12
	3.93	17	middle temporal gyrus	44	-14	-12
	3.84	15	middle frontal gyrus	-42	38	-14
	3.78	14	middle occipital gyrus	-42	-84	14
Controls-ASD	N.S					
Sad vs Baseline						
Controls	16.59	52526	cuneus	18	-72	10
	5.54	257	postcentral gyrus	64	-14	20
	4.31	48	middle temporal gyrus	-48	-26	-8
	4.3	119	postcentral gyrus	-4	-42	74
	4.02	22	lentiform nucleus	20	6	40
	3.9	35	middle frontal gyrus	26	54	-6
ASD	10.66	16445	middle occipital gyrus	54	-72	2
	4.99	472	inferior parietal lobule	54	-46	48

	4.92	46	inferior frontal gyrus	58	4	22
	4.9	289	inferior frontal gyrus	56	26	16
	4.74	259	cingulate gyrus	-4	18	36
	4.37	234	thalamus	20	-10	14
	4.19	60	lentiform nucleus	14	8	-2
	4.1	24	superior temporal gyrus	48	-18	-10
	4.07	25	superior frontal gyrus	-6	8	66
	4.06	33	inferior frontal gyrus	-38	16	-14
	3.99	33	inferior frontal gyrus	-36	28	2
	3.98	172	postcentral gyrus	64	-16	24
	3.95	20	middle frontal gyrus	-50	36	18
	3.9	15	superior frontal gyrus	12	62	-8
	3.89	53	postcentral gyrus	-26	-44	68
	3.86	47	paracentral lobule	-6	-36	72
	3.85	10	insula	46	-32	20
	3.84	25	lentiform nucleus	24	-6	-6
	3.8	26	insula	38	-4	6
	3.66	12	postcentral gyrus	-40	-38	62
ASD-Controls	4.79	12	middle occipital gyrus	54	-72	2
	4.74	91	superior temporal gyrus	-60	-42	6
	4.7	36	precuneus	-10	-62	42
	4.08	21	lentiform nucleus	-32	-24	4
	3.95	28	lingual gyrus	32	-64	-4
	3.71	12	middle temporal gyrus	-58	-40	-6
Controls-ASD	N.S					
Neutral vs Baseline						
Controls	12.87	56704	lingual gyrus	26	-62	-4
	4.98	161	medial frontal gyrus	-4	-24	72
	4.52	30	superior frontal gyrus	24	64	-10
	4.02	50	postcentral gyrus	64	-14	14
	3.76	25	superior temporal gyrus	54	-26	-4
	3.69	11	medial frontal gyrus	12	66	4
ASD	8.77	9282	lingual gyrus	28	-64	4
	6.83	1624	lentiform nucleus	-22	-2	-6
	6.58	3962	anterior cingulate	6	20	26
	5.82	1129	inferior frontal gyrus	-50	8	22
	5.81	390	thalamus	-10	-16	0

	5.6	250	superior frontal gyrus	-28	56	18
	5.44	615	inferior frontal gyrus	40	26	10
	5.11	437	precentral gyrus	-48	-2	34
	4.91	68	thalamus	20	-14	16
	4.42	101	inferior parietal lobule	-58	-36	26
	4.37	263	lentiform nucleus	18	14	-2
	4.33	64	inferior frontal gyrus	34	32	-6
	4.3	15	precentral gyrus	62	0	6
	4.15	60	medial frontal gyrus	-6	-26	70
	4.02	17	middle frontal gyrus	36	10	48
	3.96	51	superior frontal gyrus	28	52	20
	3.91	12	middle occipital gyrus	-44	-82	10
	3.85	12	fusiform gyrus	46	-44	-16
ASD-Controls	4.96	100	superior frontal gyrus	2	16	58
	4.82	38	superior frontal gyrus	-14	8	58
	4.69	221	superior frontal gyrus	-26	58	18
	4.36	39	precuneus	-8	-62	38
	3.66	22	medial frontal gyrus	-6	44	28
Controls-ASD	N.S					

Table 2: Functional connectivity between the PCC and other default network regions. The threshold was set at $p < 0.05$ (FWE corrected). Only major clusters were reported. Table 2A: Functional connectivity in the ASD group. Table 2B: Functional connectivity in the control group.

A.

Region	Brodmann's areas	Cluster size		MNI Coordinates		
			t	x	y	z
Retrosplenial	BA 30	29	10.92	-2	-46	20
		36	10.91	2	-46	20
		10	8.16	16	-38	-8
		1	6.99	12	-54	6
		2	6.82	-18	-42	-4
		1	6.82	8	-54	6
		2	6.73	-14	-38	-4
		1	6.67	16	-54	6
Left angular gyrus		55	7.31	-42	-58	36
Right angular gyrus		31	7.54	54	-60	34
Left medial prefrontal	BA 32/10	180	10.5	-24	60	-6
		159	8.68	-2	52	12
		5	6.98	-24	60	14
		3	6.91	-22	62	12
		1	6.55	-26	54	26
		3	6.45	-24	44	30
		1	6.4	-14	58	4
Right medial prefrontal	BA 32/10	348	11.1	8	68	6
		1	6.56	12	38	14
Left temporal lobe		290	9.58	-56	-6	-22
		50	8.72	-14	-60	20
		37	8.14	-36	18	-24
		41	7.7	-46	-46	-16
		28	7.31	-66	-36	-12
		8	7.17	-28	-26	-12
		32	6.93	-50	-48	10
		41	6.85	-46	-60	20
		1	6.47	-50	2	-34
Right temporal lobe		123	9.72	52	-20	-22
		181	9.65	66	-54	20
		22	8.74	38	12	-22
		32	8.56	22	-62	24
		9	7.84	18	-54	16
		6	7.35	38	20	-24
		2	6.96	26	-18	-10
		4	6.54	42	18	-32

Left superior frontal gyrus	483	11.21	-22	60	-4
	127	8.72	-16	34	34
	2	6.6	-2	48	36
	2	6.44	-16	50	28
Right superior frontal gyrus	27	9.62	36	34	38
	49	9.52	12	70	6
	119	9.02	20	30	50
	9	7.38	6	48	36
	7	7.27	10	70	10
	9	6.97	10	60	-12
	3	6.49	8	64	-20
Left parahippocampal gyrus	26	8.04	-10	-36	-2
	15	7.94	-24	-26	-10
	7	7.59	-18	-32	-4
	12	7.32	-22	-12	-20
	9	7.3	-24	-60	-8
	2	6.97	-12	-52	0
	3	6.68	-26	-8	-14
Right parahippocampal gyrus	34	8.16	16	-38	-8
	15	7.59	22	-24	-12
	2	6.89	12	-38	-6

B.

Region	**Brodmann's areas**	**Cluster size**		**MNI Coordinates**		
			t	**x**	**y**	**z**
Retrosplenial	BA 30	36	14.68	2	-46	18
		29	13.1	-2	-54	20
		57	11.19	16	-38	-8
		87	11.13	8	-54	6
		57	11.11	-18	-42	-4
		25	9.27	12	-54	6
		60	8.74	-12	-64	8
		17	7.91	-16	-60	10
		3	6.82	-2	-74	4
Left angular gyrus		212	8.61	-52	-72	30
Right angular gyrus		253	10.41	52	-58	34
Left medial prefrontal	BA 32/10	1382	14.14	-26	64	-4

		4	7.46	-42	44	0
		12	7.39	-52	52	-4
		3	7	-40	40	24
		3	6.81	-10	16	30
		1	6.46	-6	20	-10
		1	6.43	-8	14	34
Right medial prefrontal	BA 32/10	950	14.67	8	68	6
		7	9.37	18	24	42
		9	7.96	8	20	-10
		1	6.62	12	22	46
		2	6.55	12	62	30
		1	6.54	16	30	32
Left temporal lobe		41	11.04	-18	-38	-2
		362	10.63	-36	14	-30
		1194	9.84	-48	-48	-16
		103	9.46	-20	-60	16
		1288	9.41	-58	-74	16
		2	7.86	-24	-34	-4
		16	7.77	-32	-56	-8
		7	7.49	-32	-34	-12
		15	6.84	-48	-68	-20
		1	6.65	-26	-52	-2
		1	6.6	-32	-20	-10
		1	6.59	-26	-30	-8
		1	6.46	-36	-16	-26
		1	6.43	-46	-60	-16
		1	6.42	-60	-18	-6
Right temporal lobe		1511	16.62	52	-18	-22
		123	12.16	18	-54	16
		1476	10.74	56	-56	20
		64	9.78	38	20	-24
		33	8.38	24	-40	-4
		4	8.13	26	-18	-12
		68	8.06	44	-22	8
		27	7.77	34	-32	14
		58	7.58	40	2	-38
		5	7.51	30	-14	-12
		10	6.99	38	18	-34
		1	6.67	40	-26	-2
		1	6.49	26	-30	-8
Left superior frontal gyrus		1703	15.16	-22	58	2
		49	8.78	-8	46	48
		56	8.32	-30	22	54
		97	7.87	-8	30	54

	65	7.6	-12	-16	74
	17	7.41	-30	54	28
	1	6.62	-10	42	36
Right superior frontal gyrus	1972	13.36	18	30	50
	4	5.06	4	56	-20
Left parahippocampal gyrus	631	12.57	-10	-36	-2
	196	12.01	-16	0	-16
	1	6.59	-36	-18	-26
	1	6.42	-28	-12	-14
Right parahippocampal gyrus	827	11.19	16	-38	-8
	1	6.41	18	6	-18